University of Montenegro
Biotechnical faculty
Univerzitet Crne Gore
Biotehnički fakultet

Agriculture and Forestry
Poljoprivreda i šumarstvo
3

Agriculture and Forestry, Vol.66. Issue 3: 1-252, Podgorica, 2020

ISSN 0554-5579; E-ISSN 1800-9492; DOI: 10.17707/AgricultForest
COBIS.CG-ID: 3758082 www.agricultforest.ac.me

Agriculture and Forestry - ***Poljoprivreda i šumarstvo***
PUBLISHER - ***IZDAVAČ***
University of Montenegro – Univerzitet Crne Gore
Biotechnical faculty (BTF), Podgorica - Biotehnički fakultet, Podgorica
Bul. M. Lalića 1, 81000 Podgorica, Crna Gora (Montenegro), P.Box 97,
Tel.: +382 20 268434; +382 20 268437; Fax: +382 20 268432
Web: www.agricultforest.ac.me; E-mail: agricultforest@ac.me

Indexed in (by alphabethical order): AGRICOLA, AGRIS, CAB Abstracts, CAB Direct, CABI full text, COBISS, CrossRef, DOI, DOAJ, EBSCO (currently included in EDS EBSCO Discovery Service), Elektronische Zeitschriftenbibliothek EZB (Electronic Journals Library), Genamics JournalSeek, Global impact factor (GIF), Google Scholar, HathiTrustt, Index Copernicus, International Scientific Indexing (ISI), Israel Union List of Electronic Journals (ULE), JournalRate (Academic journal search tool), Journals Impact factor (JIF) by Global Society for Scientific Research (GSSR), KoBSON, MALMAD, National Agricultural Library – USA, Open Academic Journals Index (OAJI), ProQuest, Research bible, RoMEO, Regional Information Center for Science and Technology (RICeST) – Iran, Rubriq database, SCOPUS, The Open University Library, The Rudjer Bošković Institute Library, Ulrichsweb, Universal Impact Factor, WorldCat, XML, Zeitschriftendatenbank (ZDB), Zoological Record of the Clarivate Analitics Master Jouurnal List, ...

The journal "Agriculture and Forestry" is funded by the Biotechnical faculty, Co-funded by the Ministry of Science & the Ministry of Agriculture and Rural Development of Montenegro

CIP – Каталогизација у публикацији
Централна народна библиотека Црне Горе, Цетиње
ISSN 0554-5579
COBIS.CG-ID 3758082

CONTENT

Antunović, Z., Senčić, Đ., Klir, Ž., Zmaić, K., Samac, D., Novoselec, J. (2020): Organic livestock farming in Republic of Croatia- state and perspective development. Agriculture and Forestry, 66 (3): 7-13.

DOI: 10.17707/AgricultForest.66.3.01

Zvonko ANTUNOVIĆ, Đuro SENČIĆ, Željka KLIR, Krunoslav ZMAIĆ, Danijela SAMAC and Josip NOVOSELEC [1]

ORGANIC LIVESTOCK FARMING IN REPUBLIC OF CROATIA- STATE AND PERSPECTIVE DEVELOPMENT

SUMMARY

Recently, in the Republic of Croatia an increase in organic farming was observed. The aim of this paper is to present the state and perspectives of organic livestock farming development in Croatia during recent five years. There has been an increase in the number of organic farmers by 114% and the number of organic processors by 52.28%. The used agricultural land decreased by 1.54%, while the land under organic production in total used agricultural land increased, which is 6.94%. Areas sown with crops important for organic livestock feeding increased the most for rapeseed and green forage from arable land and gardens and a decrease was observed in oats. The largest increase regarding livestock was in the number of Equidae and sheep and goats, while the only decrease was observed in poultry. The largest increase was also found for sheep meat, beef and pork, while in dairy production the largest increase was in goat milk, as well as production of cheese. Expansion of organic production is observed in the recent five years in Croatia. This contributes to the growing interest in organic production, as indicated by increases in the analyzed indicators in the organic livestock sector.

Keywords: organic livestock farming; the Republic of Croatia; state and perspectives of development.

INTRODUCTION

Organic agriculture in the world has been the fastest growing branch of agricultural production in recent years. The Republic of Croatia has recorded significant increase in organic agriculture with the same trend in organic livestock farming. The reasons for the abovementioned can be seen through Croatia's outstanding natural resources as well as their conservation and the development of organic agriculture, which all contribute to the development of a country based on natural and ethical principles, without which no development is complete

[1]Zvonko Antunović, (corresponding author: zantunovic@fazos.hr), Đuro Senčić, Željka Klir, Krunoslav Zmaić, Danijela Samac, Josip Novoselec, Faculty of Agrobiotechnical Sciences Osijek, J. J. Strossmayer University of Osijek, V. Preloga 1, 31000 Osijek, CROATIA

Paper presented at the GEA (Geo Eco-Eco Agro) International Conference 2020, Podgorica.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:28/05/2020 *Accepted:23/06/2020*

(Senčić et al., 2011). Favorable climatic conditions, the possibility of producing cheap and good quality food, large quantities of floorcloth, smaller financial investments and the possession of certified organic land areas are very good prerequisites for the spread of organic livestock farming in the Republic of Croatia (Antunović, 2011; Gugić et al., 2017). The aim of organic livestock farming is to obtain high-quality food for human consumption, but also to ensure animal welfare and environmental protection (Senčić et al., 2011). Organic livestock production encourages the use of natural sources in a particular breeding area, grazing, open barns and canopies, which ensures better adaptation of animals to environmental conditions (Antunović, 2011). In the common market of the European Union, the advantages of the Republic of Croatia are, among other things, reflected in the possibility of developing organic agriculture, including organic livestock farming, and through the integration with tourism, as well as the protection of native plant and animal genetic resources and the conservation of biodiversity. According to the data of the Croatian Chamber of Economy, cited in the Ministry of Agriculture's Annual Report on the State of Agriculture in year 2018, it was pointed out that the annual value of the Croatian market of organic products is around 100 million EUR, which is certainly not negligible, while the share of consumption of organic products in total consumption is 2.2%. Thus, the aim of this paper is to present the state and perspectives of the development of organic livestock farming in the Republic of Croatia, by analyzing the period of the recent five years.

STATE AND PERSPECTIVES OF DEVELOPMENT OF ORGANIC LIVESTOCK FARMING IN THE REPUBLIC OF CROATIA

In the Republic of Croatia in the recent five years there has been an increase in the number of organic farmers by 114% and in the number of organic processors by 52.28%.

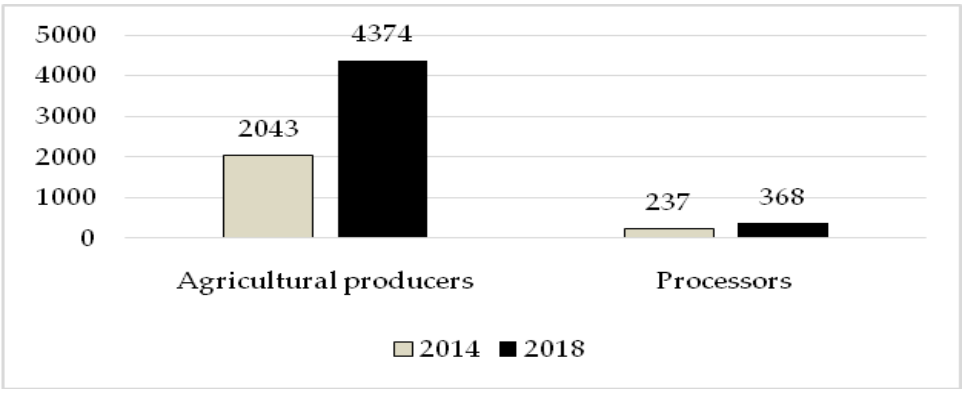


Figure 1. Number of organic agricultural entities in the Republic of Croatia in the last five years (Croatian Bureau of Statistics, 2019).

During the recent five years, the first five entities in the processing and preserving of meat and its products have been registered in the Republic of Croatia. Likewise, the number of registered producers of plant and animal oils and fats were also increased significantly by 121.62%, as well as the number of dairy producers by 37.50%.

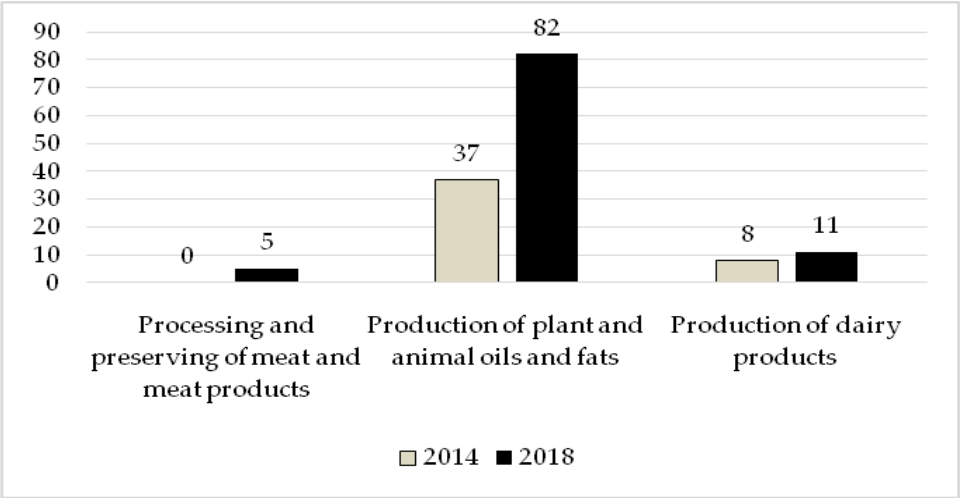


Figure 2. Number of processors of organic livestock products (Croatian Bureau of Statistics, 2019).

Table 1. The area of utilized agricultural land and area under organic production in the last five years in the Republic of Croatia (Croatian Bureau of Statistics, 2019).

Year	**Agricultural land used, (ha)**	**Areas under organic production, (ha)**	**Share of areas under organic production in total utilized agricultural area,%**
2014	1 508 885	50 054	3.32
2015	1 537 629	75 883	4.94
2016	1 546 019	93 814	6.07
2017	1 496 663	96 6185	6.46
2018	1 485 645	103 166	6.94
Difference (2014-2018), %	- 1.54	+ 51.48	

The area of utilized agricultural land in the Republic of Croatia has decreased by 1.54% in the recent five years, while the area under organic production has increased significantly from 50.054 ha to 103.166 ha, which is an increase of 51.48% (Table 1). In the recent five years there has also been an increase in the share of areas under organic production in total utilized agricultural area from 3.32% in 2014 to 6.94% in 2018. Although significant

increase of areas under organic production in total utilized agricultural area in the Republic of Croatia has been identified in the recent five years, although anticipated increase from the Action Plan of the Ministry of Agriculture has not yet been fully realized, where an increase of 8% is expected by 2016. The Ministry of Agriculture of the Republic of Croatia in the year 2011 adopted an Action plan of development of organic agriculture with the objective to accelerate the development of organic livestock farming, and to ensure supply primarily the Croatian market, and also ensure the placement of organic products abroad. According to Antunović et al. (2016) limiting factors in the development of organic agriculture are depopulation of rural areas, unfavorable educational structure, insufficient education of farmers and consumers of organic products, problems with unresolved land ownership along with numerous cropped and scattered land areas as well as mined areas.

Table 2. Total area of organic crops from arable land most commonly used in livestock feeding and their organic production in the recent five years in the Republic of Croatia (Croatian Bureau of Statistics, 2019).

Crop	**Surface, ha**					**Trend, %**
	2014	**2015**	**2016**	**2017**	**2018**	
Arable land and gardens	27 459	34 281	44 147	44 083	50 281	+83.11
Wheat and spelt	3 654	4 680	6 011	4 785	6 376	+74.49
Barley	1 081	783	1 487	1 351	1 358	+7.10
Oat	1 235	905	1 557	0 418	1 108	-10.28
Corn, dry grain	1 768	2 174	2 185	2 506	3 102	+75.80
Rapeseed	1 293	1 034	2 421	3 699	4 079	+215.47
Sunflower	1 520	1 335	1 438	2 175	2112	+38.95
Soybean	1 339	2 607	2 563	2 826	2 286	+70.72
Green forage from arable land and gardens	10 023	12 221	16 860	16 248	20 002	+99.56

Analysis of Tables 2 and 3 viewed crops (agricultural crops) used in organic farming for animal feeding which plays a significant part in composition of their diets. It can be seen that the largest increase in the area over the past five years was sown with rapeseed (by 215.47%) and the area under green forage from arable land and gardens increased by 99.56%, while the area under oats decreased by 10.28%. This has been influenced by the various incentives for organic production, and green payments. It is also evident that production has increased in all prominent crops (agricultural crops). According to the analysis of production over the past five years, the largest increase was recorded for maize (dry grain) by 395.36%, soybeans by 395.05% and wheat and spelt by 380.28%.

Table 3. Total organic production of crops (agricultural crops) in the last five years in the Republic of Croatia (Croatian Bureau of Statistics, 2019).

Crop	Production, t					Trend, %
	2014	2015	2016	2017	2018	
Arable land and gardens	71 889	102 694	149 698	149 535	144 563	+101
Wheat and spelt	4 493	11 664	19 459	17 416	21 579	+380.28
Barley	3 375	2 480	3 409	4 497	4 114	+21.90
Oat	1 474	1 777	3 437	3 184	2 206	+49.66
Corn, dry grain	4 285	11 921	18 273	17 295	21 226	+395.36
Rapeseed	2 751	2 309	4 953	8 877	8 898	+223.45
Sunflower	1 900	2 278	3 044	6 279	6 047	+218.26
Soybean	1 130	5 839	6 145	7 493	5 594	+395.05
Green forage from arable land and gardens	44 978	52 467	80 929	74 080	88 923	+97.71

Table 4 shows the number of livestock heads in organic farming by species in the recent five years in the Republic of Croatia. The largest increase was recorded in the number of Equidae (by 720.60%) and in the number of sheep and goats by 187.30% and 170.55%, respectively. The only decrease was in the number of poultry (by 26.38%). The reason for that is the inclusion of grant for organic production, as well as the inclusion of grants related to production.

Table 4. Number of heads of livestock in organic farming by species in the recent five years in the Republic of Croatia (Croatian Bureau of Statistics, 2019).

Species	Year					Trend, %
	2014	2015	2016	2017	2018	
Cattle	7 308	7 002	14 442	17 226	19 613	+ 168.38
Pigs	961	1 114	1 083	1 468	1 887	+ 96.36
Sheep	21 690	23 774	50 135	54 583	62 315	+ 187.30
Goats	1 552	2 163	3 080	3 381	4 199	+ 170.55
Poultry	2 540	2 093	3 388	2 174	1 870	- 26.38
Equidae	291	265	1 753	1 929	2 388	+ 720.60
Bee colonies	3 649	3 418	2 065	1 721	2 022	- 44.59

The analysis of the data presented in Table 5 shows a significant trend of increasing the majority of organic animal products in the past five years in the Republic of Croatia, with the exception of the organic honey, which production decreased by 48.33%. The largest increase was found for sheep meat, beef and pork. Analyzing the production of organic milk, the largest increase was in the

production of goat milk, and significant increase was observed in the production of cheese.

The increase in the production of organic animal products is also influenced by the introduction of various grants, as well as the increased interest in organic products purchase.

Table 5. Organic animal products in the recent five years in the Republic of Croatia (Croatian Bureau of Statistics, 2019).

Product, t	**Year**					**Trend, %**
	2014	**2015**	**2016**	**2017**	**2018**	
Meat*, total	109	1 200	1 689	1 627	2 185	+ 1 904.6
Beef	75	811	1 110	1 133	1 456	+ 1 841.0
Pork	10	60	91	57	141	+ 1 310.0
Sheep meat	22	316	474	424	571	+ 2 495.5
Goat meat	2	13	14	13	17	+ 750.0
Milk, total	1 782	6 000	5 163	5 783	3 094	+ 73.63
Cows´ milk	1 700	5 947	5 023	5 671	2 846	+ 67.41
Sheep milk	51	-	38	28	73	+ 43.14
Goat milk	31	40	102	84	175	+ 464.52
Cheese	4	13	8	7	43	+ 975.0
Table eggs, peaces	65 000	117 775	246 890	206 000	183 000	+ 181.54
Honey	60	48	47	29	31	- 48.33

*All kinds of meat included meat from younger categories of named animal species

For further significant development of organic livestock farming, it is crucial to address, apart from land restrictions, a difficulty in procuring good quality breeding material originating from organic farming and good quality organic feedstuffs for balancing animal rations, especially in highly demanding production stages (gravidity, lactation). Insufficient promotion, undeveloped market, misuse of the “ECO” label for the purpose of product promotion, and insufficient education of farmers and consumers of organic animal products are important facts that should be eliminated as soon as possible for the purpose of development of organic livestock farming. The perspective of organic livestock farming development in Croatia is very good. The richness of natural resources, regulated legislation on organic agriculture and incentive for the development of environmentally friendly tourism related to the revitalization of rural areas, along with initiatives for introduction of "smart villages", as well as various incentive of the Ministry of Agriculture for organic production are good support for the further development of organic livestock farming in Croatia. The large number of protected areas and the introduction of GMO-free zones by almost all Croatian counties are also advantages for the development of organic livestock farming in Croatia. In addition, there is a growing demand for organic animal products not only in Croatia but also more widely, which also contributes to the increased

interest in this production and contributes to its higher quality perspective for development.

CONCLUSIONS

An analysis of organic livestock farming in the recent five years in the Republic of Croatia shows the expansion of this production, some difficulties, and significant advantages that contribute to its overall positive image. This has also contributed to the growing interest of this production, which is indicated by significant increase of analyzed indicators, not only by farmers but also by consumers of organic animal products, as well as by those users whose perception of environmental protection and biodiversity are especially emphasized.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Antunović, Z. (2011). Organic farming of sheep and goats. In Đ. Senčić, eds. Organic zootechnique, Faculty of Agriculture in Osijek, pp. 92-122.

Antunović, Z., Novoselec, J., Klir,. Ž. (2016): Organic sheep and goat farming in the Republic of Croatia - state and development perspectives. Proceeding of the 51st croatian and 11th international symposium of agriculture, 15-18 February, Opatija, Croatia, pp. 306-310.

European Union (2007): Council Regulation (EC) No. 834/2007. on organic production and labeling of organic products with regard to organic production, labeling and control. Official Journal of the European Union [Online] (Available on-line with updates http://32007R0834) (verified 2nd 30 August 2017).

Gugić, J., Grgić, I., Dorbić, B., Šuste, M., Džepina, M., Zrakić, M. (2017): Status overview and development prospect of organic agriculture in Croatia. *Glasnik zaštite bilja*, 60: 1, 20-30.

Ministry of Agriculture (2019): Annual report. Sheep, goats, and small animals. Križevci, p. 88.

Senčić, Đ., Antunović, Z., Mijić, P., Baban, M., Puškadija, Z. (2011): Organic zootechnique. Faculty of Agriculture in Osijek, p. 239.

Annual Report on the state of agriculture in year 2018. Ministry of Agriculture, Zagreb (available at https://poljoprivreda.gov.hr/UserDocsImages/dokumenti/poljoprivredna_politika/zeleno_izvjesce/2019_11_13_Zeleno%20izvjesce2018.pdf

Action plan for the development of organic agriculture in the Republic of Croatia for the period 2011-2016 (2011): Ministry of Agriculture. Zagreb p. 37.

Ministry of Agriculture of the Republic of Croatia (available at www.mp.hr)

Bureau of Statistics (available at www.dzs.hr)

Đokić, M., Marković, B., Gantner, V., Marković, M. (2020): Association of genetic variants of β-lactoglobulin gene with milk traits of Jezeropivska sheep breed. Agriculture and Forestry, 66 (3): 15-23.

DOI: 10.17707/AgricultForest.66.3.02

Milena ĐOKIĆ[1], Božidarka MARKOVIĆ [1], Vesna GANTNER [2], Milan MARKOVIĆ [1]

ASSOCIATION OF GENETIC VARIANTS OF β-LACTOGLOBULIN GENE WITH MILK TRAITS OF JEZEROPIVSKA SHEEP BREED

SUMMARY

Association of genetic variants of β-lactoglobulin (β-Lg) on milk traits of Montenegrin autochthonous sheep breed - Jezeropivska sheep was studied. Polymorphisms within ovine β-Lg genes were detected using PCR-RFLP method by RsaI restriction endonuclease. Milk yield and milk composition parameters during lactation were determined by using the standard ICAR procedures.

The obtained results showed a prevalence of A allele (0.625) compared to B allele (0.375), while the frequencies of genotypes were: 0.364 (AA), 0.523 (AB) and 0.113 (BB). No statistically significant differences in duration of lactation and total milk yield between these three genotypes were found.

Daily milk yield was the highest (0.70 kg) in ewes with BB genotype β-lactoglobulin then AB genotype follows (0.60 kg) and AA (0.54 kg) genotype. Genotype AA of Jezeropivska breed was linked with the higher protein content (5.86%). The protein content in the milk of AB and BB genotype was the same (5.64%). Significant association between AB genotype of β-lactoglobulin gene with higher lactose content (4.67%) was found.

The results presented in this study could be useful in improvement of the concept of conservation and sustainable use of this autochthonous sheep breed, while gene of β-Lg as a potential marker in improving milk traits of sheep.

Keywords: Jezeropivska sheep, milk yield, milk composition, β-lactoglobulin, genotype.

INTRODUCTION

Milk and dairy products from sheep represent a significant part of the agricultural economy worldwide, especially in marginal areas (Selvaggi et al., 2015). There is a strong association between milk protein polymorphisms and its yield, composition and technological aspects. Hence, in-depth research of genetic

[1]Milena Đokić, (corresponding author: milena.dj1405@gmail.com), Božidarka Marković, Milan Marković, University of Montenegro, Biotechnical Faculty Podgorica, MONTENEGRO.
[2]Vesna Gantner, Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek, CROATIA.

Paper presented at the GEA (Geo Eco-Eco Agro) International Conference 2020, Podgorica
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:15/06/2020 *Accepted:04/09/2020*

polymorphisms of sheep milk proteins, especially considering the great genetic biodiversity of sheep breeds is necessary to be carried out. Milk proteins are very heterogeneous. Casein, as the main milk protein, constitutes approximately 75-80% of the total milk proteins, while whey proteins 20-25% (Davoodi et al., 2016). The whey proteins have a high nutritive value due to fact that they are a precious source of digestible proteins. The whey proteins account for 17–22% of total proteins in sheep milk. Whey obtained from sheep milk is particularly rich in β-lactoglobulin, but low in α-lactalbumin content (Moatsou et al., 2005).

The genes for β-Lg protein are located on chromosome 3 with 4.9 Kb long transcription units containing 7 exons and 6 introns. There are three polymorphic genetic variants (A, B and C alleles) of sheep β-Lg described until now (Amigo et al., 2000; Şahin et al., 2011; Rustempašić et al., 2018). The polymorphism is a consequence of the substitution of one nucleotide in exon II (T-C), which is expressed by a change in the amino acid at position 20 (Tir in His). β-Lg A has tyrosine while β-Lg B histidine (Prinzenberg and Erhardt, 1999; Amigo et al., 2000; El-Shazly et al., 2012; Özmen and Kul, 2016). The variant C is a subtype of ovine variant A with a single exchange of Arg-Gln at position 148. The C variant was described first in German Merino landsheep at a frequency of 0.175 and in Hungarian Merino crosses. In Spanish Merinos the C allele was found at low frequencies (0.013), (Prinzenberg and Erhardt, 1999; Amigo et al., 2000).

The β-Lg polymorphism was also studied to check its relationships with milk yield and milk composition. According to the recent investigations, genetic polymorphism of milk proteins (β-Lg) in sheep has been closely associated with milk production parameters and technological properties (El-Shazly et al., 2012; Özmen and Kul, 2016; Wafaa et al., 2019). Mele et al. (2007) highlighted a co-dominant effect of beta-lactoglobulin alleles on the concentration of some milk fatty acids. In the other studies of ewe's β-Lg (Staiger et al. 2010, Kawecka and Radko, 2011) contradictory results have been obtained.

Montenegro has specific geographical characteristics and the climatic conditions not favourable for intensive growing of field crops - due to that animal husbandry was a major agricultural and economic activity in the past, especially sheep sector (Marković et al., 2007; Marković et al., 2011). Natural grasslands have a special importance in Montenegro, because their share in the total agricultural area is above 90% and they are often the only source of fodder for ruminants (Dubljević et al., 2020).

The sheep production is mainly based on rearing autochthonous breeds. Jezeropivska pramenka is one of the most important autochthonous Montenegrin sheep breeds, and made about 20% of its total sheep population. The breed is traditionally reared in mountain area of Durmitor and Sinjajevina, and partly in the central part of the country. Jezeropivska breed belongs to the Pramenka group of breeds with coarse wool and long tail. It is well adapted to extreme environmental conditions, especially to harsh mountain climates. Milk production capacity of Jezeropivska breed varies a lot, depending of rearing conditions. In the study of Marković et al. (2014), average milk yield was 94.1 kg with variation

from 50 kg to 200 kg, while average duration of lactation was 178.5 days. In the same study, the percentages of the main milk components were: 5.71% of fat, 5.57% of protein, 4.47% of lactose and 11.04% of solid non-fat. Similar to the other Montenegrin sheep breeds (Bardoka, Ljaba, Sora, Sjenička and Žuja) Jezeropivska pramenka is three-purpose breed, with meat and milk as the main products, while wool is low value by-product. In Montenegro sheep milk is generally used for manufacture of different traditional local product (cheeses, yogurt, kaymak - skorup, jardum etc.). Sheep's milk has high values of chemical components, and it is the best raw material for the production of cheese, because it gives twice higher randman than cow's milk (Jandrić and Savić, 2019). Sheep milk products are of excellent quality and high nutritional value, and therefore they are highly valued on the market (Marković et al., 2016).

Having in mind the importance of autochthonous breeds in generally, the aim of this research was to do genetic characterization of polymorphic forms of β-Lg genes in Jezeropivska pramenka sheep breed. Also, for obtaining complete data, it is important to investigate milk production traits (duration of lactation, milk yield and milk composition) and to analyse the effect of β-Lg genotypes on the milk properties of this autochthonous Montenegrin sheep breed what would be in line with similar research on other sheep breeds in Europe and in the world.

The obtained data of polymorphism of milk protein gene can be used as an important selection tool in the sheep breeding programs. Effective selection on favourable genotypes of milk proteins can increase the frequency of alleles with a positive effect on milk properties and milk quality.

MATERIAL AND METHODS

The study was conducted in a typical area of rearing Jezeropivska Pramenka breed, at the Žabljak plateau. The study was carried out on 44 ewes of Jezeropivska Pramenka sheep breed. Milk yield was recorded during lactation. Milk samples to examine the chemical composition of milk were collected, while blood samples were taken to determine β-lactoglobulin polymorphism.

Milk recording was performed in accordance with ICAR rules (AT method). The first milk recording was performed 20 to 30 days after lambing and consequently in regular monthly intervals to the end of the lactation period. In total, five recordings were performed during lactation. During each of milk recording, individual milk samples were collected in special plastic bottles (50ml).

The blood samples were collected from the jugular vein of milking ewes, using blood collection tube – vacutainers with EDTA, as an anticoagulant.

Analyses of the milk chemical components (fat, protein, lactose, non-fat dry matter - SNF) were performed using the Milk Scan 4000 instrument in the Dairy Laboratory of the Biotechnical Faculty in Podgorica. Isolation of DNA from blood samples and β-lactoglobulin genotyping were performed in the Laboratory of Molecular Genetic at the Biotechnical Faculty in Podgorica.

DNA extraction: Genomic DNA was isolated from the blood samples using phenol-chloroform method (Ivanković and Dovč, 2004). The quality of DNA was checked by spectrophotometry and good quality DNA samples were used in genotyping.

PCR - RFLP of β-lg gene: β-Lactoglobulin genotypes were identified using procedure described by Ivanković and Dovč (2004) and Şahin et al. (2011). The amplification of a part genome DNA - genes for the synthesis of milk protein β-lactoglobulin (120 bp) was performed using a programmed Thermal Cycler (Eppendorf Mastercycler). The PCR amplifications were performed in reaction mixtures of 20 µL containing 10 µL of PCR Master Mix, 1,1 µL of each primer, 5,8 µL H_2O and 2 µL genomic DNA. Thermal Cycler was programmed for an initial denaturation at 95°C for 3 min, followed by 35 cycles each with denaturing at 93°C for 15 s, annealing at 58°C for 15 s, extension at 72°C for 2 s, and a final extension at 72°C for 2 min. The restriction fragments were directly analyzed by electrophoresis in 1% agarose gels in 1×TAE buffer, stained with ethidium bromide and visualized under UV light. The genotypes of the analyzed individuals at the β-lactoglobulin locus were determined using the restriction fragments observed in the gel.

Table 1. The Primer sequences for β-lactoglobulin variants

Primer β-Lg	**Primer Sequences**	**Restriction Enzymes**
BLG1	5'CAACTCAAGGTCCCTCTCCA3'	RsaI
BLG2	5'CTTCAGCTCCTCCACGTACA3'	

A total volume of 15 µL of each PCR product was digested with 10 µL of RsaI endonuclease restriction enzymes for 3 hours at 37°C. Digested products were analyzed by means of electrophoresis in 3% agarose gel stained with ethidium bromide. The digested products were visualized and documented.

Statistical analysis: The data processing was performed using the statistical program Statistica (version 10). The frequencies of alleles and β-lactoglobulin genotypes were determined by the goodness test of fit to HWE (Hardy Weinberg Equilibrium).

RESULTS AND DISCUSSION

Restriction fragment length polymorphism was evidenced after digestion with RsaI endonuclease. Allele discrimination was based on size differentiation (bp) of β-Lg and three different genotypes of Jezeropivska pramenka breed: AA (66, 37 and 17 bp), AB (103, 66, 37 and 17 bp) and BB (103 and 17 base pairs) were detected.

The results of genetic characterization (Table 2) showed high domination of allele A with frequency of 0.625 in comparison to allele B with frequency of 0.375. Regarding genotypes, the highest frequency was identified for AB genotype (0.523), then for AA genotype (0.364) and the lowest for BB genotype (0.113).

The occurrence of β-Lg variants in this study is similar to the other sheep breeds studied so far. According to the research of Ivanković and Dovč (2004), Kusza et al. (2014), El-Shazly et al. (2012) and Marković et al. (2015) A and B alleles of β-Lg were widely reported in almost all examined sheep breeds. Similar to our results for Jezeropivska pramenka breed, the higher frequencies of allele A of β-Lg were reported by Amigo et al. (2000) for Barbaresca-Siciliana, Lacaune, Tsigaja and Massese breeds, Wafaa et al. (2019) for Awassi breed, as well as Amigo et al. (2000) for many West European sheep breeds (Blackfaced, Border Leicester Merino, Cheviot, Dorset Horn, Delle Langhe, East Friesian, Finnish Landrace, Manchega, Massese, Merino, Merinoland, Segurena, Suffolk, Welsh). The highest frequency of allele A in Turkish sheep breeds was detected in Tuj breed as 0.7188 (Şahin et al., 2011).

Table 2. Frequency of genes and genotypes for β-lactoglobulin of Jezeropivska pramenka (n=44)

β-lactoglobulin		**Ho**	**He**	**Frequency**
Alleles	**A**	55	55.01	0.625
	B	33	33.21	0.375
Genotype	**AA**	16	17,19	0.364
	AB	23	20.63	0.523
	BB	5	6.29	0.113

The allele and genotypes frequencies of β-lactoglobulin in Pramenka breed sheep in Bosnia and Herzegovina, which belong to the same group as Jezeropivska breed (group of Pramenka breeds) are very similar to ours. Rustempašić et al. (2018) reported that allele frequency was 0.547 and 0.453, A and B respectively, while genotype frequencies were: AA (0.325), AB (0.445), and BB (0.229). However, the results of Masala et al. (2020) for Dupska Pramenka breed in Bosnia and Herzegovina were shown higher frequency of B genetic variation (0.52) than A (0.48), while the most common genotype was the AB determined in 68% of the examined population.

The opposite to our results, Amigo et al. (2000) reported domination of allele B for Carranzana, Chios, Lacha, Sarda, Tajik, Valle del Belice breeds, as well as El-Shazly et al. (2012) for Noemi and Sawakni breeds.

The higher frequency of AB genotype, similar to ours, was obtained for many autochthonous breeds: Croatian Pag breed ewes (Čubrić-Čurik et al. 2002); Greek Chios breeds (Triantaphyllopoulos et al. 2017); native Turkish fat-tailed sheep breeds - Akkaraman, Awassi, Tuj, Karakaş, Norduz, Güney Karaman and Kangal (Şahin et al. 2011) and in three Hungarian sheep breeds - Cokanski, Rusty Tsigai and Zomborski Tsigai (Kusza et al. 2014).

The average values with standard deviation of milk production traits (lactation duration, total milk yield, daily milk yield) for the three β-Lg genotype are presented in Table 3.

The lactation duration of Jezeropivska pramenka breed varied in the narrow range, from 173.19 days of AB genotype to the 177.23 days of AA genotype, and differences among genotypes were not significant (P>0.05). The total milk yield was lowest in AA genotype (96.34 kg) and highest in BB genotype (109.09 kg), but the differences between them were not significant (P>0.05). From the other side, the obtained results show significant differences (P<0.05) in daily milk yield, where ewes with BB genotype had the highest daily yield (0.70 kg), followed by AB genotype (0.60 kg), while AA genotype had lowest (0.54 kg).

Table 3. Association of β-Lg genotypes with milk production traits of Jezeropivska pramenka sheep breed

Milk traits	**β-Lg genotype**		
	AA	**AB**	**BB**
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD
Lactation duration, days	177 ± 23.13	173 ± 19.59	173 ± 25.46
Total milk yield, kg	96.34 ± 18.21	102.25 ± 36.42	109.09 ± 54.27
Daily milk yield, kg	0.54^{a} ± 0.21	0.60^{ab} ± 0.33	0.70^{b} ± 0.49

The average values with different letters in the superscript indicate significant differences between β-Lg genotypes (P<0.05).

The results of our research related to the association of β-Lg polymorphism with milk performance in sheep indicate the superiority of BB to the other genotypes (AA and AB) in milk yield, without statistically significant differences among these genotypes. The similar relations among genotypes were obtained for Polish Merino (Kawecka and Radko, 2011) and of East Friesian dairy sheep (Staiger et al., 2010).

Regarding milk composition, the highest fat and protein content (4.91% and 5.86%) were of AA genotype, while the lowest (4.65% and 5.64%) obtained for AB genotype. The significant differences identified between AA and the other two genotypes only for protein content (P<0.05), Table 4.

Table 4. Association of β-Lg genotypes with milk composition traits in Jezeropivska pramenka sheep breed

Milk composition	**β-Lg genotype**		
	AA	**AB**	**BB**
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD
Fat %	4.91 ± 2.53	4.65 ± 1.83	4.84 ± 2.23
Protein %	5.86^{a} ± 1.29	5.64^{b} ± 0.92	5.64^{b} ± 0.90
Lactose %	4.55^{a} ± 0.65	4.67^{b} ± 0.54	4.61^{ab} ± 0.60
SNF %	11.14 ± 1.71	11.21 ± 0.94	11.14 ± 0.68

The averages with different letters indicate significance of differences between β-Lg genotypes (P<0.05); SNF- solid non fat.

No statistically significant differences were found in milk fat content between genotypes in the studied sheep. The opposite to the fat and protein results, the lactose content was significantly lower in AA genotype (4.55%) than in AB genotype (4.67%), while content of lactose in BB genotype was in the middle (4.61%). Regarding SNF content, there were no significant differences between genotypes, the results ranged from 11.14% in AA and BB genotype to 11.21% in AB genotype.

Our results of association of β-Lg genotypes and protein and lactose content in milk are in accordance with results of Kusza et al. (2014) revealed for different dairy breeds, but not fully with the results reported by Triantaphyllopoulos et al. (2017), who found that AA genotype of β-Lg gene of Chios and Karagouniko breeds was associated with the highest fat, protein percentage, while greater lactose percentage observed in genotype AB - 5,42% compared to genotype AA - 4.96% and to genotype BB - 5.32%.

The results of investigation of Özmen and Kul (2016) on allele and genotype effects on milk production traits Sakiz and Awassi breeds are fully in accordance with our results. They obtained that ewes of AB genotype had a greater milk yield than those with AA genotype, while homozygous AA ewes had higher milk protein and fat percentage when compared with other two genotypes.

CONCLUSIONS

This study shows that Jezeropivska sheep breed has a genetic variability in the β-lactoglobulin locus. A significant association of β-Lg genotypes and daily milk yield and milk quality was revealed. Although, the results showed that milk yield did not have a clear relationship with β-LG genotype, protein and lactose content were statistical significant. More investigation of the effect of β-Lg polymorphism and milk composition is needed. It was determined that milk protein polymorphism can be considered as a potential tool in the breeding program of Jezeropivska sheep breed, especially regarding selection of genotypes associated with better milk composition. This research is also the important contribution for creation of long-term conservation strategy of the autochthonous sheep breeds in Montenegro.

REFERENCES

Amigo, L., Recio, I., Ramos, M. (2000): Genetic polymorphism of ovine milk proteins: its influence on technological properties of milk- a review. International Dairy Journal 10, 135-149.

Čubrić-Čurik, V., Feligini, M., Lukac-Havranek, J., Curik, I., Guiseppe, E. (2002): Genetic polymorphism of β-lactoglobulin in native sheep from the Island of Pag. Food Technol Biotechnol. 40: 75-78.

Davoodi, S. H., Shahbazi, R., Esmaeili, S., Sohrabvandi, S., Mortazavian, A.M., Jazayeri, S. and Davoodi, A.T. (2016): "Health-Related Aspects of Milk Proteins." Iranian journal of pharmaceutical research : IJPR vol. 15,3 (2016): 573-591.

Dubljević, R., Radonjić, D., Marković, M. (2020): Production traits of major types of grasslands in the Durmitor area. Agriculture and Forestry, 66 (2): 229-236

El-Shazly, S.A., Mahfouz, M.E., Al-Otaibi, S.A., and Ahmed, M.M. (2012): Genetic polymorphism in β-lactoglobulin gene of some sheep breeds in the Kingdom of Saudi Arabia (KSA) and its influence on milk composition. African Journal of Biotechnology Vol. 11(19), 4330-4337.

Ivanković, A. and Dovč, P. (2004): Polimorfizem genov za β-laktoglobulin in αs1-kazein paske ovce, Acta agriculturae slovenica, 842, 121- 130.

Jandrić, M., Savić, Z. (2019): Influence of milk on the content and changes of milk fat during production of sjenički white cheese. Agriculture and Forestry, 65 (4): 175-182. DOI:10.17707/AgricultForest.65.4.15

Kawecka, A and Radko, A. (2011): Genetic polymorphism of β-lactoglobulin in sheep raised for milk production. Journal of Applied Animal Research, Vol. 39, No. 1., 68-71.

Kusza, S., Sziszkosz, N., Nagy, K., Masala, A., Kukovics, S. and András, J. (2014): Preliminary result of a genetic polymorphism of β-lactoglobulin gene and the phylogenetic study of ten balkan and central european indigenous sheep breeds. Biohimica polonica. Vol. 62, No 1/2015, 109-112.

Marković, B., Đokić, M., Kostić, S., Marković, M. (2016): Stanje i trendovi ovčarske proizvodnja na durmitorskom području, CANU, Podgorica.

Marković, B., Ivanković, A., Mirecki, S, Marković, M. (2015): Genetic polymorphism of beta – lactoglobulin in the three sheep breeds in Montenegro. International symposium on Animal Science, Novi Sad, Serbia. 159-164.

Marković, B., Marković, M., Adzić, N. (2007): The farm animal genetic resources of Montenegro, Biotechnology in Animal Husbandry 23 (3-4) ISSN 1450-9156, Institute for Animal Husbandry, Belgrade-Zemun, 1-9.

Marković, B., Marković, M., Mirecki, S., Radonjić D., (2014): Variation of milk yield and milk composition of Pivska pramenka sheep breed through lactation, Proceedings of the International Symposium on Animal Science, Beograd-Zemun, 167-173.

Marković, B., Marković, M., Radonjić, D., Veljić, M. (2011): Sustainable sheep and goat production based on the local breeds in Montenegro. 8th Global Conference on Consevation on Animal Genetic Resources. Tekirdag Turkiye. 414-420.

Masala, A., Alilović, I., Ohran, H., Kusza, S., Goletic, T., Hrkovic-Porobija, A., HodzicGenetic, A. (2020): Polymorphism β-Lactoglobulin Gene in Dubska Pramenka Sheep Breed. CMBEBIH 2019 , 641-645.

Mele, M., Conte, G., Serra, A., Buccioni, A., Secchiari, P. (2007): Relationship between beta-lactoglobulin polymorphism and milk fatty acid composition in milk of Massese dairy ewes. Small Ruminant Research 73, 37–44.

Moatsou, G., Hatzinaki, A., Samolada, M., Anifantakis, E. (2005). Major whey proteins in ovine and caprine acid wheys from indigenous Greek breeds. Int. Dairy J. 15: 123–131.

Özmen, O., Kul, S. (2016): Investigating the genetic polymorphism in the exon 2 region of ovine beta-lactoglobulin gene and its association with some milk traits. Ankara Üniv Vet Fak Derg, 63, 323-328.

Prinzenberg, M. and Erhardt, G. (1999): Molecular genetic characterization of ovine b-lactoglobulin C allele and detection by PCR-RFLP. J. Anim. Breed. Genet. 116, 9–14.

Rustempašić, A., Dokso, A., Zečević, E., Hodžić, A., Hrković-Porobija, A., Sarić, Z., Miskoska–Milevska, E., Popovski, Z. and Brka, M. (2018): Polymorphism of β-lactoglobulin in Pramenka sheep breed in Bosnia and Herzegovina.

Şahin, E., Karsli, T., Elmaci, C., Balcioğlu, M.S. (2011): Beta-Lactoglobulin Gene Types in Turkish Fat-Tailed Sheep Breeds. Short communication. Kafkas Univ Vet Fak Derg 17 (6): 1031-1033.

Selvaggi, M., Laudadio, V., Dario, C., Tufarelli, V. (2015): β-lactoglobulin gene polymorphisms in sheep and effects on milk production traits: A review. Adv. Anim. Vet. Sci. 3(9): 478-484.

Staiger, E. A., Thonney, M. L., Buchanan, J. W., Rogers, E. R., Oltenacu, P. A. and Mateescu, R.G. (2010): Effect of prolactin, β-lactoglobulin, and κ-casein genotype on milk yield in East Friesian sheep. American Dairy Science Association. J. Dairy Sci. 93, 1736–1742.

Triantaphyllopoulos, K.A., Koutsouli, P., Kandris, A., Papachristou, D., Markopoulou, K.E., Mataragka, A., Massouras, T., Bizelis, I. (2017): Effect of βlactoglobulin gene polymorphism, lactation stage and breed on milk traits in Chios and Karagouniko sheep breeds, Annals of Animal Science, DOI: 10.1515/aoas-2016-0058.

Wafaa, I. I., Hassooni, H. A. and Alkhazraji, W. J. (2019): Association of β-lactoglobulin gene polymorphism with milk production and composition in local Awassi sheep. Plant Archives Vol. 19, Supplement 2, ISSN:0972-521

Steiner, Z., Prakatur, I., Novoselec, J., Samac, D., Klir, Ž., Antunović, B., Benak, S., Gantner, V., Ronta, M. (2020): Influence of pumpkin seed cake on production and slaughter indicators for broiler chickens. Agriculture and Forestry, 66 (3): 25-31.

DOI: 10.17707/AgricultForest.66.3.03

Zvonimir STEINER[1], Ivana PRAKATUR[1], Josip NOVOSELEC[1], Danijela SAMAC[1], Željka KLIR[1], Boris ANTUNOVIĆ[1], Stipo BENAK[2], Vesna GANTNER[1], Mario RONTA[1]

INFLUENCE OF PUMPKIN SEED CAKE ON PRODUCTION AND SLAUGHTER INDICATORS FOR BROILER CHICKENS

SUMMARY

The aim of the research was to determine the possibility of adding pumpkin seed cake (PSC) in feed mixtures for broiler chickens, as well as its influence on production and slaughter indicators. The experiment consisted of control and two experimental groups of broiler chickens fed for 6 weeks with feed mixtures containing different rates of PSC (0%, 7% and 14%, respectively). Statistically significantly higher body mass was determined in the E1 group compared to the E2 group ($P<0.01$) and in the E1 group compared to the C group ($P<0.05$). Regarding the percentage shares of different body parts in body weight, the C and E1 groups had statistically very significantly ($P <0.01$) higher values of wing shares compared to the E2 group. Regarding the skin color, L value was statically significantly ($P<0.05$) higher in the C group, compared to the E1 group. From all of the above, it is to conclude that PSC in the rate of 7% in fodder mixtures can be recommended for feeding broilers in the period from the 1st until the 42nd day of age, with a potential to improve production and slaughter indicators.

Keywords: PSC, feeding, broilers, slaughtering indicators.

INTRODUCTION

According to the data of the Croatian Bureau of Statistics, the total number of farming poultry heads in the Republic of Croatia in the period from 2012 until 2016 was in average 10 million. Nutritional needs of broiler chickens are very high. In average, feed mixtures contain amino acid ratio and high crude protein level, ranging from 37% to 48%. As the price of feed participates within the total production cost in the rate of 70%, it is in the interest of farmers to lower this cost. Furthermore, the price of conventional fodders is often unfavourable for producers, so alternative fodders are being sought (Steiner et al., 2015).

[1]Mario Ronta, (corresponding author: mronta@fazos.hr), Zvonimir Steiner, Ivana Prakatur, Josip Novoselec, Danijela Samac, Željka Klir, Boris Antunović, Vesna Gantner, Faculty of Agrobiotehnical Sciences Osijek 1, CROATIA.
[2]Stipo Benak, Belje plus d.o.o. CROATIA
Paper presented at the GEA (Geo Eco-Eco Agro) International Conference 2020, Podgorica.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:29/05/2020 *Accepted:30/07/2020*

Alternative fodders intended for broiler production are divided to those rich in crude proteins and those rich in energy. PSC is an alternative fodder that has a potential to be a source of protein in poultry due to high levels of crude proteins (Wafar et al., 2017). Pumpkin seed cake (PSC) contains up to 500 g/kg of crude proteins and 70 g/kg of raw fibre. For the purpose of preparation of meals for monogastric animals, only the pellets and cakes from shelled seeds are being used (Domaćinović 2006).

Fodder produced from shelled seed can be used in feeding swine and older poultry, but despite of large amount of protein, due to the lysine deficiency, it is being used in addition to other protein fodders (Dumovski and Milas, 2004). PSC is a high-quality by-product rich in proteins, derived from pumpkin oil production (Antunović, 2015). Zdunczyk et al. (1999) studied chemical composition and nutritive value of PSC from Cucurbita pepo compared to soybean meal. The content of crude protein and ether extract was higher in PSC compared to soybean meal (598.0 vs. 474.2 g/kg and 124.6 vs. 28.3 g/kg, respectively).

The main fatty acids found in PSC were oleic acid (50.4%) and linoleic acid (29.9%). The proteins found in PSC contained significant amounts of tryptophan (1.54 g/16 g N). In contrast, lysine content (3.21 g/16 g N) and isoleucine content (3.83 g/16 g N) were low. Furthermore, small amounts of phenolic compounds (2.61 g/kg), low activity of trypsin inhibitor (1.33 TUI/mg) and small amounts of α-galactoside (19.9 g/kg) were found in PSC. Among the anti-nutritive compounds, only the content of inositol phosphate in PSC (40.5 mg/g) was higher than in soybean seed (10.8 mg/g). The simultaneous coefficient of digestibility (TD) of PSC protein was similar (83.1% vs. 83.5%), but the protein efficiency ratio (PER) was lower compared to the soybean meal (1.01 vs. 1.50). Kreft et al. (2002) point out that PSC represents better source than pumpkin seed or pumpkin oil. Also, PSC is a high quality source of high quality fatty acids, proteins, herbal sterols and vitamins.

MATERIAL AND METHODS

The study included 135 one-day-old broilers of Ross 308 chickens divided into three groups; Control group (C), Experimental group 1 (E1), and Experimental group 2 (E2). The C group fed feed mixtures with no PSC added. The E1 and E2 groups of broilers fed fodder mixtures containing 7% and 14% of PSC, respectively.

The feeding itself took place in two phases; the initial – "starter" phase, which lasted for the first 21 days of broiler chickens' life and the final – "finisher" phase, which lasted from 22nd until 42nd day of broiler chickens' life. Feeding and watering of chickens was ad libitum. In order to examine the influence of PSC on production and slaughter indicators of broiler chickens, the recipes presented in Table 1 were prepared.

Table 1. Raw material composition of fodder mixtures

Ingredients %	C group		E1 group		E2 group	
	Starter 0-21 d	Finisher 22-42 d	Starter 0-21 d	Finisher 22-42	Starter 0-21 d	Finisher 22-42 d
Corn meal	37.2	41.3	41.7	44.2	45.8	48.4
Barley meal	11.5	10	11.5	11.5	11.5	11.5
Wheat meal	5	5	5	5	5	5
Soybean meal	20	20	20	20	18.4	15.4
Pumpkin seed cake (PSC)	0	0	7	7	14	14
Soybean meal	21	17	9.5	5.5	0	0
Plant oil	1.5	3	1.5	3	1.5	1.9
Monocalcium phosphate	1	1	1	1	1	1
Limestone	1.5	1.4	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premixture	1	1	1	1	1	1
Crude protein	21.07	19.88	21.16	20.00	21.16	20.00
ME (kcal/kg)	3000	3112	3005	3105	3027	3087
Ca (g)	0.92	0.87	0.90	0.89	0.90	0.88
P (g)	0.61	0.59	0.60	0.58	0.60	0.58

During the experiment, individual weekly weighing of broiler chickens was performed, together with simultaneous monitoring of consumption of feed by groups. Based on the obtained results, weekly weight gains and conversion values in broiler chickens were calculated. After 42nd day of age and after 10-hour of hunger, 10 broiler chickens from each group were sacrificed randomly. For the purpose of this study, the trunks of broiler chickens were weighed and then subtracted to the basic parts; drumstick with thighs, wings, chest and back with pelvis. Each of mentioned body parts were separately weighed. Based on the obtained values, the proportion of basic parts in the trunk was determined and expressed in absolute values (g).

The dressing percentage of chicken carcasses was calculated as the difference between finishing and slaughter weight, and was expressed as a percentage of slaughter weight (%) in relation to the final fattening mass. For the purpose of testing meat quality, the pH1 and pH2 values were measured in chest muscles (within 45 minutes and 24 hours after slaughter, respectively) using the digital pH meter - Mettler MP 120-B. The colour (L* - for the degree of lightness,

a* - for the degree of redness and b* - for the degree of yellowness) of meat and skin of chickens was determined with Minolta Chromametar CR-410.

The results of the research were analysed by the usage of computer program Statistica 12 (2013). The differences between mean values were tested by GLM (General Linear Model) variance analysis (ANOVA) at significance levels $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Table 2 represents the weight gain (g) in broiler chickens per week. It is apparent that the groups C and E1 had statistically very significantly ($P<0.01$) higher weight gain compared to the E2 group in the 1st week of fattening period. In the 2nd week, the trend continued, but without statistically significant differences. In the 3rd week of the experiment, the E1 group had statistically very significantly ($P<0.01$) higher weight gain compared to the groups C and E2. Within the 4th week, the E1 group had statistically very significantly ($P<0.01$) higher weight gain compared to the E2 group. In the 5th week of the experiment, the E1 group had the highest weight gain and together with the C group statistically very significantly ($P<0.01$) higher weight gain compared to the E2 group. In the 6th week, the E1 group had statistically very significantly ($P<0.01$) higher weight gain compared to the C and E2 groups.

The obtained results are consistent with the results of Janječić et al. (2016), who investigated the influence of adding PSC to fodder mixtures at rates of 5% and 10%. Wafar et al. (2017) also found that addition of PSC should not have negative impact on growth rates, although they observed a different trend. In their experiment, increasing of the share of PSC (10%, 15% and 20%) was followed by the increase of total weight gain, while in the group fed by the feed mixture with the lowest level of PSC (5%), statistically significant ($P<0.05$) lower weight gains were determined.

Table 2. Weight gains (g) in different weeks of fattening broiler chickens

Week	C group		E1 group		E2 group		P value*
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
1st	104.5^{A}	3.6	105.2^{A}	4.6	100.2^{B}	4.0	0.000000
2nd	169.6	36.0	168.8	29.2	157.2	28.2	0.123654
3rd	283.1^{A}	39.9	307.7^{B}	42.5	271.1^{A}	26.6	0.000048
4th	356.6	179.1	400.9^{A}	82.6	308.7^{B}	28.9	0.001837
5th	496.6^{A}	105.1	536.1^{A}	96.3	428.7^{B}	63.3	0.000001
6th	578.5^{A}	102.2	718.8^{B}	168.6	604.2^{A}	100.0	0.000001

PSC – pumpkin seed cake

The feed conversion (kg/kg) per weeks and divided by groups is presented in Table 3. It is apparent that in the 1st week of fattening, the E1 group had

slightly higher conversion (1.1 kg/kg) compared to the C group (1.08 kg/kg), while the E2 group had the lowest conversion (1.05 kg/kg). In the 2nd and 3rd weeks, the highest feed consumption per kg of body weight gain had the E2 group, whilst the lowest values were found in the C group. In the period from the 4th week until the end of the experiment, the highest feed consumption was found in the E2 group, whilst the lowest feed consumption was found in the C group. Overall, the groups E1 and E2 had slightly higher feed consumption per kg of body weight (2.08 kg/kg) compared to the C group (1.95 kg/kg).

The obtained results are partially consistent with the results of Janječić et al. (2016), where feed consumption was the highest in the group fed with 10% PSC in fodder mixture, which in the same time had the lowest body mass. The group fed fodder mixture with 5% of PSC had the lowest consumption of feed per kg of growth, together with lower final body mass than the control group. Martinez et al. (2010) did not notice differences in conversion between the groups. Wafar et al. (2017), obtained results consistent with the results of this experiment

Table 3. Conversion of feed mixture (kg/kg) in broiler chickens

Week	C group	E1 group	E2 group
1st	1.08	1.10	1.05
2nd	1.09	1.12	1.21
3rd	1.46	1.67	1.81
4th	1.81	1.93	1.90
5th	1.99	2.09	2.03
6th	2.33	2.44	2.37
Total	1.95	2.08	2.08

PSC – pumpkin seed cake

Table 4 presents the differences related to the slaughtering indicators between the groups. The group E1 had statistically very significantly ($P<0.01$) higher final body mass compared to the other two groups. The masses of carcasses were the highest in the E1 group in the following way: statistically very significantly higher ($P<0.01$) than in the E2 group and statistically significantly higher ($P<0.05$) than in the C group. The dressing percentage was the highest in the C group (79%) and the lowest in the E1 group (76.5%). There were no statistically significant differences between the groups. The weight of drumsticks with thighs was the highest in the E1 group (565 g) on statistically very significant level ($P<0.01$) compared to the E2 group (448 g). The breast weight was the highest in the E1 group (752 g), statistically very significantly ($P<0.01$) higher in relation to the E2 group (584 g) and statistically significantly higher ($P<0.05$) compared to the C group (665.5 g). The wings' weight was statistically very significantly ($P<0.01$) higher in the E1 group (234 g) than in the other

groups (E2 = 209 g, C = 210 g). The back weight was statistically very significantly (P<0.01) higher in the E1 group (340 g) compared to the C group (315.5 g) and the E2 group (258 g). The weight of the neck was the highest in the E1 group (127 g) and statistically very significantly (P<0.01) higher compared to the E2 group (101.5 g).

The obtained results are consistent with the results of Aguilar et al. (2011) who noticed increased values in the experimental groups fed 3.3% and 6.6% of PSC in fodder mixtures, while the group fed 10% of PSC had lower values of slaughter indicators. Wafer et al. (2017) and Martinez et al. (2010) did not obtain differences in slaughter indicators.

Table 4. Slaughter indicators of broiler chickens

Indicator	C group		E1 group		E2 group		P value
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
Live weight (g)	2295.9^{A}	239.3	2636.7^{B}	225.9	2078.3^{A}	113.2	0.000005
Carcass weight (g)	1813.5^{a}	200.3	$2018.0^{A.b}$	190.1	$1600.5^{b.B}$	117.4	0.000052
Dressing percent %	79	9.4	76.5	6.6	77.01	7.9	0.430796
Drumstick with thigh weight (g)	508.5	62.9	565.0^{A}	58.4	448.0^{B}	42.1	0.000280
Brest weight (g)	664.5^{a}	82.8	$752.0^{b.A}$	74.5	$584.0^{b.B}$	50.4	0.000062
Wings' weight (g)	210.0^{A}	8.2	234.0^{B}	15.8	209.0^{A}	12.0	0.000106
Back weight (g)	315.5^{A}	57.4	340.0^{A}	44.0	258.0^{B}	29.0	0.001166
Neck weight (g)	115.0	20.7^{B}	127.0^{A}	18.3	101.5^{B}	4.7	0.005994

CONCLUSIONS

Based on the obtained results, it can be concluded that PSC can be used as a substitute of quality for soybean meal in feeding broiler chickens. However, there are certain limitations related to quantity. Better results were achieved with smaller share (7%) of PSC in fodder mixture. After adding higher levels of PSC (14%), a bit worse production results were achieved, both in growth rates and in slaughtering indicators.

REFERENCES

Antunović, Z., Novoselec, J., Sičaja, V., Stainer, Z., Klir, Ž., & Matanić, I. (2016). The use of pumpkin seed cake in the diets of lambs in organic farming. Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme, 57(1), 3-9.

Aguilar, Y. M., Yero, O. M., Navarro, M. I. V., Hurtado, C. A. B., López, J. A. C., & Mejía, L. B. G. (2011). Effect of squash seed meal (Cucurbita moschata) on broiler performance, sensory meat quality, and blood lipid profile. Brazilian Journal of Poultry Science, 13(4), 219-226.

Domaćinović, M. (2006). Hranidba domaćih životinja. Poljoprivredni fakultet u Osijeku.

Dumovski, F., Milas, Z, (2004). Priručnik o proizvodnji i upotrebi stočne hrane – krme. Hrvatsko agronomsko društvo, Zagreb.

Državni zavod za statistiku https://www.dzs.hr/ 13. 04. 2018

Janječić, Z.,Bedeković, D., Pintarić, J., Babić-Alagić, J. (2016): Upotreba pogače sjemenki bundeve u hranidbi brojlera. Zbornik sažetaka 23. međunarodnog savjetovanja Krmiva 2016. 1-2 lipnja., Opatija, Hrvatska, str 80.

Kreft, I., Stibilj, V., & Trkov, Z. (2002). Iodine and selenium contents in pumpkin (Cucurbita pepo L.) oil and oil-cake. European Food Research and Technology, 215(4), 279-281.

Martínez, Y., Valdivié, M., Martínez, O., Estarrón, M., & Córdova, J. (2010). Utilization of pumpkin (Cucurbita moschata) seed in broiler chicken diets. Cuban Journal of Agricultural Science, 44(4), 387-392.

Statisitica-Stat Soft, Inc. Version 12, 2013, www.statsoft.com

Steiner, Z., Domaćinović, M., Klarić, I., Ronta, M., Bertić, V., Steiner, N., & Šerić, V. (2015). Procjena upotrebe ddgs-a u krmnim smjesama tovnih pilića. Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme, 56(4), 163-167.

Wafar, R. J., Hannison, M. I., Abdullahi, U., & Makinta, A. (2017). Effect of Pumpkin (Cucurbita pepo L.) Seed Meal on the Performance and Carcass Charac teristics of Broiler Chickens. Asian Journal of Advan ces in Agricultural Research, 2(3), 1-7.

Zdunczyk, Z., Minakowski, D., Frejnagel, S., & Flis, M. (1999). Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. Food/Nahrung, 43(6), 392-395.

Dudić, B., Dudić, Z., Pejanović, R., Škatarić G., Saxunová D., Peráček T. (2020): The rural policy of Slovakia after joining the EU. Agriculture and Forestry, 66 (3): 33-51.

DOI: 10.17707/AgricultForest.66.3.04

Branislav DUDIĆ[1], Zdenka DUDIĆ[2], Radovan PEJANOVIĆ[3], Goran ŠKATARIĆ[4], Darina SAXUNOVÁ[5], Tomáš PERÁČEK[5]

THE RURAL POLICY OF SLOVAKIA AFTER JOINING THE EU

SUMMARY

The main players in rural development are for instance, factors as impact of technologies, development of domestic and foreign markets, benefits in communication and transport and population migration. The research object of the study is the rural policy and its tools such a financial subsidy contributing to rural economic development in Slovak conditions. The objective of the scientific study is to examine the development of rural policy in Slovakia since 2004 and its tools for augmentation of economic development sustaining ecologically clean environment and agricultural production. As a member state of the European Union, the Slovak Republic has the possibility to use the supporting policy instruments for the development of rural areas, agriculture and society as such. The instrument for this goal is the Rural Development Program 2014-2020. The program belongs to documents of national nature and represents a rural development strategy through various measures. Measures are grouped in line with the axes such as: increasing the competitiveness of the agricultural and forestry sector, improving the environment, the quality of life in rural areas and the diversification of the rural economy to which it is directed assistance exclusively from the European Agricultural Fund for Rural Development.

Keywords: rural development, agriculture, subsidy, common rural policy.

INTRODUCTION

The total budget for agriculture and rural development represents about 46% of EU spending per year. Agriculture and rural development has become again a central issue and a major determinant of EU development. Agricultural

[1]Branislav Dudić, (corresponding author: branislav.dudic@fm.uniba.sk), Comenius University, Faculty of Management, Bratislava, SLOVAK REPUBLIC; University Business Academy, Faculty of Economics and Engineering Management, NOVI SAD, SERBIA.
[2]Zdenka Dudić, University Business Academy, Faculty of Economics and Engineering Management, NOVI SAD, SERBIA.
[3]Radovan Pejanović, University of Novi Sad, SERBIA.
[4] Goran Škatarić, National parks of Montenegro, Podgorica, MONTENEGRO;
[5] Darina Saxunova, Tomáš Peráček, Comenius University, Faculty of Management, Bratislava, SLOVAK REPUBLIC;
Paper presented at the GEA (Geo Eco-Eco Agro) International Conference 2020, Podgorica.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:20/05/2020 *Accepted:07/08/2020*

and rural development policies play a vital role in the territorial, economic and social cohesion of the European Union and in the protection of the environment. Forms of agricultural entities where the agricultural production is conducted varies. Cooperative farming is frequently observed especially in small countries or as a complement in larger countries. „Cooperative is a group of people that allow several people or legal entity working together on a basic of voluntary held a job to improve the lives of its members (Sagimun, 2005; Amelia & Ronald, 2018). In the past there were cooperative farms in Slovakia, nowadays it is a mix of farming enterprising entities, cooperatives and micro, small and medium entrepreneurs as producers in agriculture. As a member state of the European Union, the Slovak Republic has the possibility to use supporting policy instruments for the development of rural areas, agriculture and society as such. The population exceeds 5.4 million and consists of mostly of Slovaks and the biggest Hungarian nationality in the southern territories of Slovakia. Slovakia's territory spans about 49,054 square kilometers (19,000 sq mi) and is mostly mountainous. The capital and largest city is Bratislava and the second largest city is Košice.

In the period of fifteen years from 2004-2018, Slovakia, as a member of the EU, benefitted from around 35 billion euros of EU funding. Slovakia will have €12 billion available in period between 2021 and 2027. Slovakia's contribution to the EU budget is the amount of €646 million. Slovakia is a recipient of €2.66 billion in EU funding. Financial resources obtained for financing from the EU are invested into the following areas: Agriculture (21.27%), Regional policy - cohesion and structural funds (74.72%), Research and development (3.21%), Citizenship - freedom, security and justice (0.40%), Administration (0.40%).

Slovakia is divided into 8 regions, such as Bratislava, Trnava, Nitra, Trenčín, Banská Bystrica, Žilina, Prešov and Košice region. Of the total area of Slovakia, according to the individual types of regions, the largest share of 59% spreads also in the prevailing rural areas, with the transitio-nal regions having a 36.8% share, predominantly the smallest 4.2% share being urban regions.

METHODOLOGY

Rural areas represent about 92% of the territory in the EU-28. These regions produce around 45% of gross value added (HPH) in the EU-28 and represent more than half of jobs. Rural development must be able to meet market requirements and ensure adequate food quality and protection as well as the protection of environmental policy. The research object is the rural policy of the Slovakia from historical perspective in the period after Slovakia's accession to the EU so are rural policy tools such a financial subsidy contributing to rural economic development in Slovak conditions. The objective of the scientific study is to examine the development of rural policy in Slovakia since 2004. The research approach applied when examining research phenomena was a combined approach by mixing several classical research methods (synthesis, deduction,

analysis, comparison) and empirical results obtained by processing secondary data from database of the Ministry of Agriculture of the Slovak Republic, European Commission etc. Results of the research is systemization of the rural development process and compilation of the existing organizations focusing on supporting and managing the process of rural development. Their role is to bring benefits for augmenting the quality of the life in rural areas, highlighting the ecological and environmental friendly activities (Spalevic et al, 2017a) leading to competitive food products and health improvement. These are attractive features to attract people again for living in the countryside and work as farmers. To assist to perform this old profession could result in reviving rural areas economically and in sociable, cultural and generational aspects.

CURRENT STATE OF THE KNOWLEDGE IN THE LITERATURE

Agriculture represents an indispensable production process that is beneficial substantially to every society and economy (Johnston and Mellor, 1961; Melovic et al., 2020a; Sinabell, 2009) and remains an essential driver of the rural areas of the European Union (Bournaris et al., 2016). The enterprise value of an agricultural operation is the present value of expected future cash inflows reduced by present cash outflows or future returns plus the value of future investment opportunities. A farm that maintains the flexibility to alter production in the future will be worth more than an identical farm that cannot (Dixit & Pindyck, 1994; Carey & Zilberman, 2002). The transitional years during which an agricultural producer waits for newly grafted or planted agricultural products to mature cannot be reversed. Forgone revenue cannot be recovered. The farmer as a producer has a choice to make a decision about the change in the future point. The level of a grower's perception of uncertainty about future price premiums will largely dictate the duration of the delay of an investment (West, 2019; Melovic et al., 2020b).

In big agricultural countries, an example is from in Indonesia, Muftiadi (2018) made a research in food crops market. The market is very large, he highlighted its slow growth, and pointed out that contribution to economy indicated a negative trend in rice production, the results showed a very sharp decrease during 1971-2008, the efficiency of other food crops subsector production (beans, corn, vegetables and fruits and other food crops) shows an increase in efficiency. Both subsectors tend to be more inefficient in the future, if no fundamental government policy to support the sector is accepted. It is an eternal problem that agriculture industry is underfinanced, since the prices of the product cannot fly up, as it is the essential commodity for the people’s life. Many examples prove that farmers survive in business only with the support of subsidies. (Loizou et.al., 2019) stressed that the impact of the financial crisis, being spread globally, again strengthened its effect on agriculture in Europe. Especially in south European countries where agriculture plays a more important role than in the north European countries. They point out that „the role of agriculture particularly within the reformed Common Agricultural Policy (CAP)

should be revaluated, as a chance to remunerate the knowledge and to depict its significance and potential as an economic growth factor“ (Loizou et al., 2019).

Priorities and requirements for rural development are protecting environmental policy, meeting market requirements, ensuring adequate food quality and protection. The main factors having impact on rural development are for instance, impact of technologies, development of domestic and foreign markets, benefits in communication and transport and migration of the population (El Mouatassime et al., 2019; Parsipour et al, 2019). Three key areas are important in rural development policy: the agri-food sector, the rural economy together with the rural population and the environment. (Loizou et al., 2019) demonstrated in their research that the impact of the new CAP is not limited to the primary sector, but it directly and indirectly affects other sectors, as well as the total output, employment and household income of the region. Results suggest that agriculture is an important driver of growth throughout the region (Loizou et al., 2019). Argued that regional characteristics played a critical role on the direct and indirect effects of the CAP on family and subsistence farming in the Czech Republic, Latvia, Hungary, Poland, Slovakia and Romania (Baumet et al., 2006). Stress essential commensalism of the law via necessity of high-quality legislation to secure a safe business environment for attracting investment to Slovakia (Novackova et. al, 2018). Attracting investment, even from abroad may contribute to increasing competitiveness of Slovak products.

SLOVAK AGRICULTURE INDUSTRY IN FIGURES

Structure of agricultural land in Slovakia is shown in Table 1. The Slovak Republic covers an area of 4,903,347 ha, the agricultural land is on 1,895,500 ha, of which arable land is 71%, TTP and meadows 28% and permanent crops 1%.

Table 1: Acreage of the land in the SR, in ha.

Indicator	Ø 2012 – 16	2016	2017	Index 2017/2016	Index 2017//Ø 2012 – 2016
Utilised agricultural land	1,922,277	1,918,878	1,910,654	99.57	99.40
including: arable land	1,352,614	1,347,293	1,342,885	99.67	99.28
permanent grassland 1)	18,850	17,788	17,761	96.84	94.22
home gardens	32,253	32,357	32,329	99.91	100.24
permanent meadows and pastures	518,547	521,441	517,679	99.28	99.83

Source: Statistical Office of the SR

Note: 1) vineyards, wine-growing areas under remediation, orchards, hops, other permanent crops (NAFC-RIAFE)

Slovak agriculture has still been undergoing gradual restructuring. As for agricultural primary production, the structure of businesses consists mainly of commercial farms of legal entities–trading companies (TC), agricultural

cooperatives (AC) and physical entities (PE)–especially self-employed farmers (SEF), sole proprietors – who also work as SEF and free business on a marginal basis. In the case of Slovak legal entities, an average farming area is approximately 500 ha, in the case of small farmers it is 21 ha. In Slovakia the small-size private properties represent less than one third of the private forests (Bouriaud et al., 2013). In Slovakia, there are many sole-proprietors with a very small area of land, which, however, are not significant from a societal point of view, but they supply local markets (representing more shadow economy).

Table 2: Agricultural land

Agricultural land	Legal entities	Individuals	Total
Hectares (ha)	1,529,083	366,417	1,895,500
in percent (%)	80.7	19.3	100.0

Source: Green report, 2017

Table 3: Interval of agricultural land

Interval of agricultural land	Number of enterprises		Profit/Loss (revenues-expenses)			Profit/Loss (revenues-expenses-personal income)		Income costs	
	2016	2017	2016	2017	Index	2016	2017	2016	2017
in € per ha									
up to 50	340	267	67.5	120.1	177.9	−263.6	−276.2	97.6	95.2
50 – 100	267	228	59.7	63.0	105.5	−56.4	−58.0	93.9	94.3
101 – 500	401	404	49.5	37.2	75.2	8.4	−5.6	95.9	96.8
above 500	41	45	57.6	48.6	84.4	47.7	37.5	95.6	96.8
Total	1049	944	53.9	46.6	86.5	−7.1	−12.2	95.9	96.4

Source: NAFC-RIAFE

Economic outturn and the number of SEF enterprises divided into intervals showing agricultural land intervals in € per ha of agricultural land, index in %. (Information sheets of the MARD SR, CD of NAFC-RIAFE).

Majority of the banks offer credit products for the agricultural sector and its enterprises can use:

-short-term bridging loans for the financing of operating needs up to the amount of eligible direct payments from the Agricultural Paying Agency (APA),

-short-term bridging loans for the financing of a project before a subsidy is granted from the EU structural funds,

-investment loan for the purchase of land in the territory of the Slovak Republic intended for agricultural production,

-investment mid-term and long-term loan for the procurement of tangible and intangible assets, the construction of operational premises, the reconstruction of real estate or the purchase of technologies, machines and equipment,

-loans for the financing of temporary storage of harvest in compliance with the provisions of the Act on Warehouse Receipt in the form of a term or revolving loan,
-investment loans for the purchase of shares in agricultural land. (Green Report, 2017)

ORGANISATIONS FOR RURAL DEVELOPMENT

A. European Network for Rural Development (ENRD) serves as a hub for exchange of information on how Rural Development policy, programs, projects and other initiatives are working in practice and how they can be improved to achieve more.

The ENRD is not a membership organization. Its work aims to engage and reach anyone with an interest in and commitment to rural development in Europe.

The main stakeholders of the ENRD include:

- National Rural Networks (NRNs);
- RDP Managing Authorities and Paying Agencies;
- Local Action Groups (LAGs);
- European organizations;
- Agricultural advisory services;
- Agricultural and rural researchers

B. Ministry of Agriculture and Rural Development of the Slovak Republic is responsible for the coordination of legal relations in providing any assistance or subsidies for rural development. This ministry carries out financial management and decision-making on project assignments and direct support.

C. Agricultural Paying Agency is a payment agency providing administrative activities in securing support and subsidies as a state administration body. Another important body that is involved in providing financial resources is, according to § 9 Act 280/2017, a payment agency, which is a budgetary organization linked in financial relations to the budget of the Ministry of Agriculture. This agency acts as a state administration body. The payment agency carries out activities related to the provision of support in agriculture and subsidies for rural development only on the grounds of accreditation granted by the Ministry of Agriculture.

Table 4: Budget Ministry of Agriculture and Rural Development of Slovakia for 2019-2020.

Budget 2019-2020	2019	2020
EU budget	1,236,686,967	1,107,894,383
Slovakia budget	170,220,032	173,622,521
Other	85,432,368	85,432,368
TOTAL	1,492,339,367	1,366,949,272

Source: Own elaboration based on Slovak Ministry of Agriculture and Rural Development, 2018.

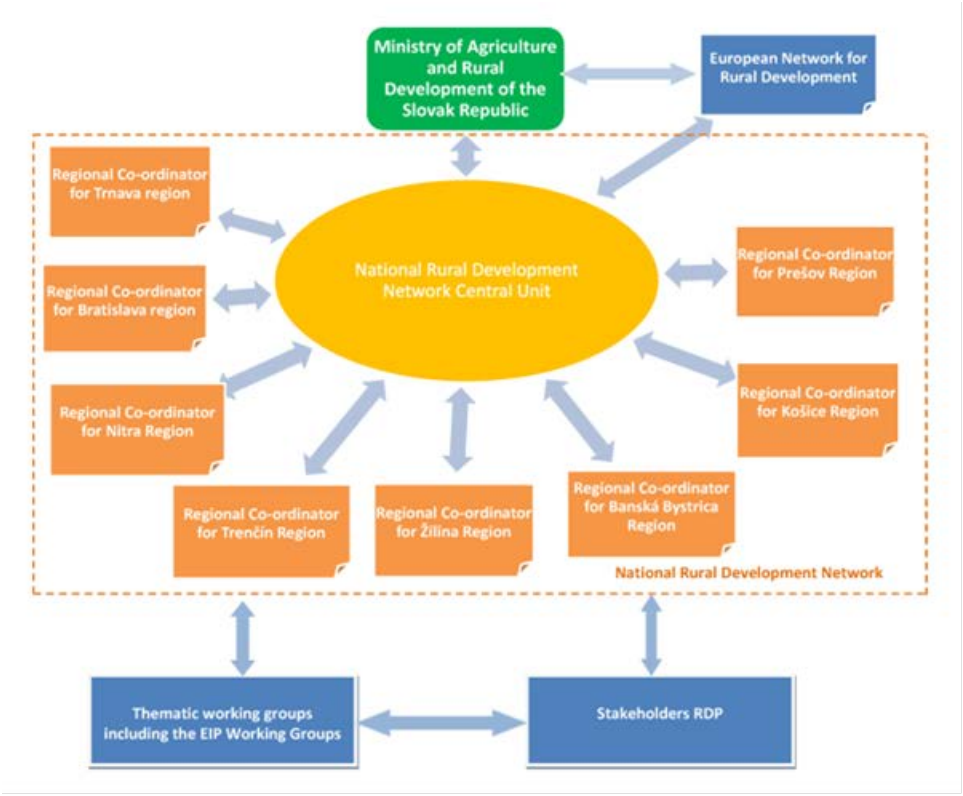


Figure 1. The structure of the NRN and the network central unit
Source: http://www.nsrv.sk/?pl=17

D. National Network for Rural Development collects and disseminates information on rural development, provides advice to potential and existing businesses. The Rural Development Agency (ARVI) is a national, contributory organization of the Slovak Ministry of Agriculture and Rural Development. It assists to collect, analyze and disseminate rural development provides feedback to responsible authorities in relation to rural development. Moreover, it promotes sustainable activities for balanced, socio-economic development of rural settlements, provides advice to potential and existing businesses, the public and non-profit sector in rural areas on their rural development activities. A regional representation is established in each region. National Rural Network (NRN) has a central office–National Support Unit hosted by the Agency for Rural Development in Nitra. Regional antennae of NRN operate in 8 regions of Slovakia, in Bratislava, Trnava, Nitra, Trenčín, Banská Bystrica, Žilina, Prešov and Košice region. The chapter 17.2 of the Rural Development Program of the Slovakia 2014–2020 defines the structure of the NRN and the network central unit (the executive body of the network).

PROGRAMS FOR THE DEVELOPMENT OF AGRICULTURE AND RURAL DEVELOPMENT BEFORE EU MEMBERSHIP AND NOW

The largest beneficiary of state aid in the agricultural sector are France (14.9%), Germany (14.2%), Spain (10.9%) and Italy (10.9%). Slovakia received 0.9% of total EU subsidies. Slovak agriculture has been still undergoing gradual restructuring. Since Slovakia has become a member of the European Union, it has the possibility to use the support policy tools for the development of rural areas, agriculture and society as such. In Slovakia the representation of the rural area amounts to 86% of the total area. The observation period can be divided into five phases:

I.	**II.**	**III.**	**IV.**	**V.**
1990-2004	2004-2006	2007-2013	2014-2020	2021-2027

Transition of Slovak Agriculture for the period 1990-2004

Slovakia's agriculture has been exposed to the external pressure of market forces for achieving greater efficiency, performance and adapting to market conditions during ten years of the economic reform in the period from 1990 to 1999. The greatest decline in agriculture was in 1993, followed by moderate growth. The decline in the rural sector and the decline in agricultural cooperatives has greatly affected the rise in unemployment. Structural Funds of the EU– SAPARD, PHARE ECOSOC (Economic and Social Cohesion) were not sufficiently utilized, only 20% of funds were used.

Rural development program 2004 – 2006

The guiding principles are those of decentralization of responsibilities - thus strengthening subsidiarity and partnership - and flexibility of programming based on a 'menu' of 22 measures (extended to 26 with the mid-term review of the Common Agricultural Policy) to be targeted and implemented according to Member States' specific needs. As a coherent package of measures, it has three main objectives: (1) To create a stronger agricultural and forestry sector, the latter recognized for the first time as an integral part of the rural development policy; (2) To improve the competitiveness of rural areas; (3) To maintain the environment and preserve Europe's rural heritage.

Slovak rural development program 2004 – 2006

On May 1, 2004, Slovakia became a member of the EU, which allowed her to use funds from the EU funds. The EU provides financial support to the agricultural and forestry sectors and rural areas to improve efficiency in agricultural production and quality of life of rural population.

Table 5: program 2004 – 2006.

Year	2004	2005	2006
In EUR	119,500,000	133,100,000	144,500,000

Source: own elaboration

"EU Quality Policy" or "Quality brand SK"

The aim of this project is to attract Slovak consumers to quality domestic products. Any Slovak producer may obtain the Slovak certificate of the quality-Slovak brand if its products are made from domestic raw materials in accordance with the declared technological procedure, parameters of quality and safety of food. It is also necessary to register raw materials in order that the total consumption of raw materials may be at least 75% of the consumption of domestic raw materials. The stages of the production process must be carried out in the territory of the Slovak Republic and products that have had excellent quality properties before will receive the Slovak certificate of quality it for a period of three years and for seasonal products for a period of one year. All criteria must be in accordance with national legislation of the SC, as well as EU legislation.

Rural development program 2007 – 2013

In the framework of the EU rural development program, Member States received €96 billion. The aims of the policy have been simplified and clarified around three clearly defined economic, environmental and territorial objectives, namely: (1) improving the competitiveness of agriculture and forestry; (2) improving the environment and the countryside; and (3) improving the quality of life in rural areas and encouraging diversification of economic activity.

Slovak rural development program 2007 – 2013

PRV 2007-2013 was approved by the European Commission decision C (2007) 6164 of December 4th, 2007 by the European Commission in Brussels (the EC assessed the program as harmoniously balanced and uniform for the whole of Slovakia). The main focus in the program, besides investing in primary

agricultural production and manufacturing, is to promote the environment and disadvantaged areas.

Table 6: program 2007 – 2013

	EU budget	Slovakia budget
SR 2007-2013	€1,969,418,078	€593,167,836
Total in € (EUR)	€2,562,585,914	

Source: own elaboration

Rural Development Program in Slovakia 2007 - 2013

OS 1: Increase in the competitiveness of agriculture and forestry sector
OS 2: Improvement of the environment and landscape
OS 3: Quality of the life in rural areas and rural economy diversification
OS 4: Leadership

Table 7: Program 2007 – 2013.

Operational system	Public subsidy			Share of OS in % (from EAFRD)
	Total	EAFRD	Slovakia	
	€	€	€	
Os 1	847,577,149	628,241,695	219,335,454	31,46%
Os 2	1,270,188,674	1,007,199,039	262,989,635	50,44%
Os 3	343,956,872	256,646,440	87,310,432	12,85%
Os 4	79,013,206	62,582,542	16,430,664	3,13%
Technical assistance	56,317,816	42,238,362	14,079,454	2,12%
NSRV – operating costs	666,666	500,000	166,666	-
NSRV – action plan	2,000,000	1,500,000	500,000	-
Total	2,599,720,383	1,998,908,078	600,812,305	100%

Source: Rural Development Programme of the Slovak Republic 2007 – 2013

Based on the results achieved, the program has largely met its objectives. Support for the investment program has helped increase gross value added, profit, economic performance and employment. As far as improving the quality of innovation and products in the agricultural, food and forestry sectors is concerned, this program has not achieved its objective.

Rural Development Programme of Slovakia 2014-2020

Rural Development Policy as a part of Common Agricultural Policy (CAP) of the European Union serves for reaching sustainable rural development. From the

European Commission perspective, the legislative proposal is drafted in a way that CAP would decisively contribute to the implementation of the strategy "Europe 2020 – A strategy for smart, sustainable and inclusive growth".

Objectives of RDP pf Slovakia 2014-2020

Rural development in the European Union countries in 2014-2020 is funded by the European Agricultural Fund for Rural Development and pursues the following objectives:

- the most important strategic goal of the programme is strenghtening the competitiveness of agricultural sector (agriculture, forestry and food industry).
- the second strategic goal is sustainable management of natural resources and adaptation to climate change.
- the third strategic goal is achieving a balanced territorial development of rural economies and communities including job creation and maintenance.

Priorities of RDP of SR 2014-2020

1. Knowledge transfer in agriculture and forestry (transversal priority)
   - Human capital and smart networking in agriculture and forestry
   - Innovation and the knowledge base of agriculture and forestry
   - Strengthening the links between agriculture and forestry / research and development
2. Competitiveness of agriculture and farm viability
   - Restructuring of farms facing major structural problems
   - Generational renewal in the agricultural sector
3. Food chain organization and risk management in agriculture
   - Integrating primary producers into the food chain
   - Supporting farm risk management
4. Preserving and enhancing ecosystems dependent on agriculture and forestry
   - *With a focus on:* biodiversity/landscapes, water, soil management and carbon sequestration
5. Resource efficiency and the transition to a low carbon economy in the agri-food and forestry sectors
   - Production of renewable energy in agriculture and forestry
   - Reducing nitrous oxide and methane emissions from agriculture
6. Job creation and the renewal of rural area:
   - Diversification, fostering creation of small businesses and job creation
   - Fostering local development in rural areas (LEADER)
   - Promotion and use of ICT in rural areas[2]

[2] http://www.apa.sk/en/project-supports

Slovak rural development program 2014-2020

The Rural Development Program of the Slovak Republic 2014 - 2020 (hereinafter referred to as the "RDP") is a program document of the Slovak Republic for the use of assistance from the European Agricultural Fund for Rural Development (hereinafter "EAFRD") in the area of achieving sustainable development of the Slovak Republic, which creates conditions for strengthening the competitiveness of agricultural and the balanced territorial development of rural economies and communities, including sustainability of job creation.

Table 8: program 2014 – 2020

	EU budget	Slovakia budget	Total
SR 2014-2020	1 574 569 461,50	524 630 234,50	2 099 199 696,00

Source: own elaboration

CAP (COMMON AGRICULTURAL POLICY) 2021 – 2027[3]

The European Union shapes its budget for a pragmatic, modern, and long-term planning for the 2021-27 period to deliver on issues that matter to Europeans. The Commission proposes that funding for the CAP is moderately reduced – by around 5% – due to less contribution, with a future union of 27 members. Based on nine objectives, the future CAP will continue to ensure access to high-quality food and strong support for the unique European farming model.

Nine objectives comprise relevant areas for farmers, which are the tasks and challenges, such as:

a) ensuring fair income for farmers due to the significance of the agricultural production and sustainability of the sector and sustainability of jobs in agricultural industry,

b) increasing competitiveness of agricultural products will help to achieve higher quality of these products,

c) rebalancing power in food chain, securing an appropriate share of all components in agricultural production,

d) encouraging climate change action, e) enhancing environmental care,

f) preserving landscapes and biodiversity – all mentioned tasks will lead to healthier products and also, they will motivate farmer to ecological production in farming (Spalevic et al, 2017b),

g) supporting generational renewal with the objective of attracting young generation to start business in this industry, that will result in

h) enhancing vibrant rural areas,

i) finally striving to protect food and health quality.

The new way of working will also entail:

[3] https://ec.europa.eu/info/food-farming-fisheries/key-policies/common-agricultural-policy/future-cap_en

1. streamlining administrative processes: countries shall submit only one strategic plan covering direct payments, rural development and sectorial strategies,
2. making environmental protection easier: through a set of standards and objectives at EU level, each country shall adapt environmental and climate actions to the reality on the ground,
3. simplifying support to young farmers: a single strategic plan will enable a consistent action for generational renewal covering both direct payments and rural development. Moreover, young farmers will have easier access to complementary income and installation support as EU eligibility criteria will be reduced.

Figure 2. Nine (9) goals for high-quality food & strong support for the European farming model.
Source: https://ec.europa.eu/info/food-farming-fisheries/key-policies/common-agricultural-policy/future-cap_en

As shown in Table 9. France would remain the largest Common Agricultural Policy recipient (17.5%), followed by Spain (12.3%). Germany (11.5%), Italy (10.2%) and Poland (8.5%).

Table 9: CAP allocation per Member State in millions (in constant 2018 prices).

Member State	EAGF – Direct payments	EAGF – Market interventions	EAFRD – Rural Development	Total amount allocated	%
Austria - AT	4,135.6	91.0	2,988.8	7,215.5	2.3%
Belgium - BE	3,020.8	2.6	417.9	3,441.3	1,1%
Bulgaria - BG	4,930.2	172.8	1,752.4	6,855.4	2.2%
Croatia - HR	2,207.7	76.7	1,750.1	4,034.5	1.3%
Czech Rep - CZ	5,218.2	44.0	1,609.7	6,871.9	2.2%
Cyprus - CY	290.8.	28.8	99.5	419.1	0.1%
Denmark - DK	5,263.5	1.8	471.6	5,736.9	1.8%
Estonia - EE	1,102.4	0.9	546.6	1,650.0	0.5%
Finland - FI	3,169.0	1.2	1,816.6	4,986.8	1.6%
France - FR	44,464.1	3,385.1	7,522.4	55,371.6	17.5%
Germany - DE	30,003.0	263.5	6,185.0	36,424.5	11.5%
Greece - EL	12,668.8	391.0	3,170.0	16,229.8	5.1%
Hungary - HU	7,587.8	200.6	2,589.1	10,377.4	3.3%
Ireland - IE	7,240.5	0.4	1,646.4	8,887.3	2.8%
Italy - IT	22,146.8	2,262.1	7,902.2	32,311.1	10.2%
Latvia - LV	1,967.4	2.0	729.7	2,699.2	0.8%
Lithuania - LT	3,343.9	3.7	1,214.2	4,561.7	1.4%
Luxemburg - LU	199.9	0.2	76.5	276.5	0.1%
Malta - MT	28.0	0.1	75.9	104.1	0.1%
Netherlands- NL	4,378.5	1.8	455.0	4,835.4	1.5%
Poland - PL	18,859.5	31.3	8,198.2	27,088.9	8.6%
Portugal - PT	3,741.0	1,038.6	3,068.1	7,847.7	2.5%
Romania - RO	11,869.7	323.0	6,006.1	18,198.8	5.8%
Slovenia - SI	802.8	34.2	636.1	1,473.1	0.5%
Slovakia - SK	2,444.5	36.6	1,416.3	3,897.5	1.2%
Spain - ES	29,750.3	2,921.7	6,228.2	38,900.2	12.3%
Sweden - S	4,187.7	3.7	1,316.0	5,507.4	1.7%
TOTAL MS	235,022.2	11,319.4	69,861.7	316,203.3	100%
% expenditure allocated	74.3%	3.6%	22.1%	100%	--

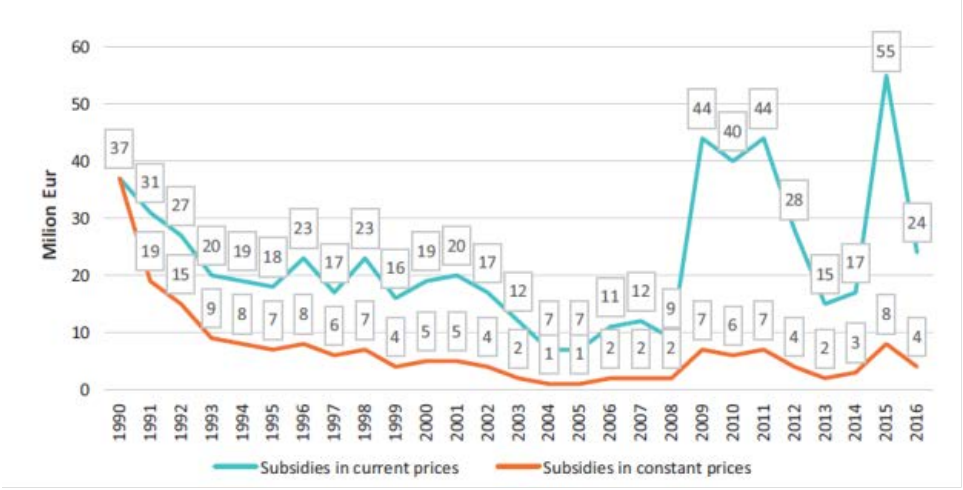
Source: Towards the Common Agricultural Policy beyond 2020: comparing the reform package with the current regulations.

RESULTS AND DISCUSSION

Public funding in 2016 subsidized the forest sector by €27,263,000 from various public sources (state budget, EU funds, etc.). In 2015, public sources allocated to the sector €58,009,000. The decrease in funding was caused by lower funding from the Rural Development Program of the Slovak Republic (RDP SR) 2014-2020 which represented the main source of public funding in forestry in

2016, funding from the RDP SR 2014-2020 was mostly secured by non-state forest enterprises. From other sources (other than the Ministry of Agriculture and Rural Development of Slovakia, funding was made available to the Military Forests and Estates of the Slovak Republic. The history of public funding (years 1990-2016) is demonstrated in Figure 3.

Figure 3 Public Funding



Source: Green Report , 2017.

Tax income represents a substantial source of public finance in contemporary economies. Tax income contributes to the public finance budget, resources of which will be used for essential and inevitable public consumption (Saxunova et.al. 2017, 2018). Therefore, it is important if industry contributes in the state budget by tax incomes.

To illustrate the example of economic benefits from agricultural sector, we show it on the example of its subsector “forestry”. It is necessary to mention that in 2016 this sector contributed to the state and municipal budgets in taxes by the amount of €52.87 million, with the VAT - the value added tax (balance of tax on inputs and outputs), as the biggest contributor - €29.53 million, or 55.9 percent of the total tax. Similarly, in 2017, VAT in taxes was in €36.24 million and represented 61.8% of the total tax. In 2016 the state and municipal (council) budgets increased by the amount of €58.61 million from VAT revenues. Compared to 2016, it increased by €5.74 million, almost equally for both state and non-state forest enterprises. The taxes have grown by €9.54 million since 2010. Property tax annually grew by 5.7%; the growth was more apparent in state enterprises in 2016. Income tax decreased by 5.6% in 2016. (See table 11). Income tax reached in 2017-€12.62 million (has been decreasing over the last 3 years); property tax came to €9.15 million, the largest sum since 2010. (Green report, 2017 and 2018).In 2016, earnings and revenue from the forestry sector amounted to the level of €508.26 million, growing by 3.4% compared to 2015.

Other earnings and revenue represent income from the trading of forest products, transplants, by-products, hunting, tourism and forest services as well as income from leasing and the sale of forest property and revenue from capital and bonds.

The contribution to the state budget by taxpayers is demonstrated in table 10, where we may see that VAT is showing a high increase from the year 2016 to 2017 after introducing electronic system of VAT monthly reports. Increasing as a total by 36%.

Table 10: Tax contribution to national and municipal budgets (million €)

Type of TAXES		2010	2012	2013	2014	2015	2016	2017
Δ in % = change in		% from year $_x$ to year $_{x+1}$, x=2010						
VAT	**State**	**17,25**	**18,26**	**18,00**	**17,01**	**18,28**	**18,13**	**21,94**
	Δ in %	1,00	5,86	- 1,42	- 5,50	7,47	- 0,82	21,01
	Non-State	**13,31**	**13,20**	**13,23**	**14,78**	**12,32**	**12,40**	**14,30**
	Δ in %	1,00	- 0,83	0,23	11,72	- 16,64	0,65	15,32
	Total	**30,56**	**31,46**	**31,73**	**31,79**	**30,60**	**29,53**	**36,24**
	Δ in %	1,00	2,95	0,86	0,19	- 3,74	- 3,50	22,72
	State	**5,38**	**5,94**	**7,14**	**6,57**	**6,35**	**6,73**	**6,88**
PRO-	Δ in %	1,00	10,41	20,20	- 7,98	2,44	5,98	2,23
PERTY	**Non-State**	**1,50**	**1,60**	**1,93**	**1,90**	**2,00**	**2,10**	**2,27**
TAX	Δ in %	1,00	6,67	20,63	- 1,55	5,26	5,00	8,10
	Total	**6,88**	**7,54**	**9,07**	**8,47**	**8,35**	**8,83**	**9,15**
	Δ in %	1,00	9,59	20,29	- 6,62	- 1,42	5,75	3,62
	State	**0,57**	**0,53**	**0,56**	**0,55**	**0,31**	**0,18**	**0,20**
ROAD	Δ in %	1,00	- 7,02	5,66	- 1,79	- 43,64	- 41,94	11,11
TAX	**Non-State**	**0,32**	**0,40**	**0,42**	**0,41**	**0,40**	**0,30**	**0,40**
	Δ in %	1,00	25,00	5,00	- 2,38	- 2,44	- 25,00	33,33
	Total	**0,89**	**0,93**	**0,98**	**0,96**	**0,71**	**0,48**	**0,60**
	Δ in %	1,00	4,49	5,38	- 2,04	- 26,04	- 32,39	25,00
	State	**5,67**	**8,14**	**4,87**	**6,82**	**8,68**	**7,79**	**7,43**
INCO-	Δ in %	1,00	43,56	- 40,17	40,04	27,27	- 10,25	- 4,62
ME	**Non-State**	**5,06**	**6,30**	**5,55**	**5,73**	**6,19**	**6,24**	**5,19**
TAX	Δ in %	1,00	24,51	- 11,90	3,24	8,03	0,81	- 16,83
	Total	**10,74**	**14,44**	**10,42**	**12,55**	**14,87**	**14,03**	**12,62**
	Δ in %	1,00	34,45	- 27,84	20,44	18,49	- 5,65	- 10,05
	State	**28,87**	**32,87**	**31,07**	**30,95**	**33,62**	**32,83**	**36,45**
TOTAL	Δ in %	1,00	13,86	- 5,48	- 0,39	8,63	- 2,35	11,03
	Non-State	**20,19**	**21,50**	**21,13**	**22,82**	**20,91**	**20,04**	**22,16**
	Δ in %	1,00	6,49	- 1,72	8,00	- 8,37	- 4,16	10,58
	Total	**49,07**	**54,37**	**52,20**	**53,77**	**54,53**	**52,87**	**58,61**
x=2010	Δ in %	1,00	10,80	- 3,99	3,01	1,41	- 3,04	10,86

Source: (Green report, 2018)

CONCLUSIONS

The preparation of the Slovak Republic for its accession to the European Union: the strategy of agricultural policy had begun to converge with the tasks and objectives of the agro-policy within the EU. The European Union has sought to help farmers be self-sufficient in the EU, to increase the share of investment in agricultural production and augment efficiency. The strategic interest of the European Union is the food safety and food self-sufficiency.

Support for rural development in Slovakia is based primarily on its ability to provide a range of public services that go beyond food production alone and on the ability of the rural economy to generate new sources of income and employment while preserving the culture, environment and heritage of rural areas. Rural development is implemented through EU financial instruments and through operational programs where the emphasis is on eliminating regional disparities and on using natural resources to develop entrepreneurship in rural areas. Great emphasis is placed on the development of rural tourism and on the use of land, forests and pastures for business. There is a room for establishing social enterprises, which are not-for-profit private organizations providing goods and services directly related to their explicit aim to benefit the community from economical and social area (Saxunova, Schurmann, 2015; Melovic et al, 2019; Nacka et al, 2019).

REFERENCES

Agricultural Paying Agency. (2018). [Online]. [Retrieved September 20. 2018]. http://www.apa.sk/en/project supports

Amelia. N.. & Ronald. N. (2018). Efforts to enhance active cooperatives in Indonesia based on macroeconomic variables. *International Journal of Economic Policy in Emerging Economies*. 11(4). 411.

Baumert J., Kunter M., Blum W., Brunner M. (2006). Teachers' Mathematical Knowledge, Cognitive Activation in the Classroom, and Student Progress. *American Educational Research Journal.* 47. 1.

Bouriaud, L., Nichiforel, L., Weiss, G., Bajraktari, A., Čurović, M., Dobsinska, Z., Glavonjic, P., Jarský, V., Sarvasova, Z., Teder, M. (2013): Governance of private forests in Eastern and Central Europe: An analysis of forest harvesting and management rights; Annals of forest research, 56(1) pp: 199-215

Bournaris, T.,Moulogianni, C.,Arampatzis, S.,Kiomourtzi, F.,Wascher, D., & Manos, B. (2016). A knowledge brokerage approach for assessing the impacts of the setting up young farmers policy measure in Greece. Environmental Impact Assessment Review, 57, 159-166. doi:10.1016/j.eiar.2015.12.004 .

Carey. J. M.. & Zilberman. D. (2002). A Model of Investment under Uncertainty: Modern Irrigation Technology and Emerging Markets in Water. *American Journal of Agricultural Economics. 84*(1). 171-183. doi:10.1111/1467-8276.00251

Dixit. A. K.. & Pindyck. R. S. (1994). Investment under uncertainty. Princeton (New Jersey): Princeton University Press.

El Mouatassime, S., Boukdir, A., Karaoui, I., Skataric, G., Nacka, M., Khaledi Darvishan, A., Sestras, P., Spalevic, V. (2019). Modelling of soil erosion processes and runoff for sustainable watershed management: Case study Oued el Abid Watershed, Morocco. *Agriculture and Forestry*, 65 (4), 241-250.

European Commission. (2018). [Online]. [Retrieved November 20. 2018]. http://europa.eu/rapid/press-release

European Commission. 2018. *Strategy for the Western Balkans [online]*. In ec.europa.eu. [cited 2018 – 03 - 25]. Available from Internet: https://ec.europa.eu/commission/news/strategy-western-balkans-2018-feb-06_sk

European Network for Rural Development. (2018). [Online]. [Retrieved November 20. 2018]. https://enrd.ec.europa.eu/about/brief_en

Green Report (report on agriculture and food sector in the Slovak Republic 2016). (2017), (2018). [Online]. [Retrieved September 20. 2018]. http://www.mpsr.sk/index.php?navID=122?start

Johnston B., Mellor J. (1961). The American Economic Review. *American Economic Association.* 51. 4. https://www.jstor.org/stable/i331432

Loizou E., Karelakis Ch., Galanopoulos K., Konstadinos Mattas K. (2019). The role of agriculture as a development tool for a regional economy. *Agricultural Systems*. 173. 482-490.

Melovic, B., Cirovic, D., Dudic, B., Vulic, T. B., & Gregus, M. (2020b). The Analysis of Marketing Factors Influencing Consumers' Preferences and Acceptance of Organic Food Products—Recommendations for the Optimization of the Offer in a Developing Market. *Foods*, 9(3), 259.

Melovic, B., Dabic, M., Rogic, S., Durisic, V., & Prorok, V. (2020a). Food for thought: Identifying the influential factors that affect consumption of organic produce in today's youth. *British Food Journal*, 122(4), 1130-1155.

Melovic, B., Milovic, N., Backovic-Vulic, T., Dudic, B., & Bajzik, P. (2019). Attitudes and Perceptions of Employees toward Corporate Social Responsibility in Western Balkan Countries: Importance and Relevance for Sustainable Development. *Sustainability*, 11(23), 6763.

Muftiadi. A. (2018). Food crops production efficiency analysis in Indonesia in 1971-2008. *International Journal of Economic Policy in Emerging Economies.* 11(3). 282. doi:10.1504/ijepee.2018.093952

Nacka, M., Simonovska, A., Skataric, G., Dudic, B. (2019): Opportunities to profit under competitive market conditions. *Agriculture and Forestry*, 65 (4): 161-174.

Nováčková. D.. Paškrtová. L.. & Saxunová. D. (2018). Investment aid as a catholicon for regional development in Slovakia? *International Journal of Economic Policy in Emerging Economies*. 11(3). 209.

Parsipour, H., Popovic, S., Behzadfar, M., Skataric, G., Spalevic, V. (2019): Cities expansion and land use changes of agricultural and garden lands in peri-urban villages (case study: Bojnurd). *Agriculture and Forestry*, 65(3): 173-187

Saxunova D.. Novackova D.. and Kajanova J.. 2018. "International Tax Competition Complying with Laws: Who are its Beneficiaries? ". *Journal of Eastern Europe Research in Business and Economics*. Vol. 2018. Article ID 145385. DOI: 10.5171/2018.145385

Saxunová D.. Nováčková D.. Kajanova J. 2017. International tax competition and tax exemption as an economic investment stimulus - The case of Slovakia. 1. ed. ISBN 978-0-9860419-7-6. - Norristown : International business information management association. 2017. - S. 2636-2648 [USB-key]

Saxunová, D., Schurman,, H., The potencial of social services. In: FAIMA Business & Management Journal. - Vol. 3, Iss. 3 (2015), s. 60-71. - ISSN 2344-4088

Sinabell F. (2009). A spatial econometric analysis of the regional growth and volatility in Europe. *Austrian Economic Association*. 36. 2. 193-207.

Spalevic, V., Lakicevic, M., Radanovic, D., Billi, P., Barovic, G., Vujacic, D., Sestras, P., & Khaledi Darvishan, A. (2017a). Ecological-Economic (Eco-Eco) Modelling in the River Basins of Mountainous Regions: Impact of Land Cover Changes on Sediment Yield in the Velicka Rijeka, Montenegro. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 45(2), 602-610.

Spalevic, V., Radanovic, D., Skataric, G., Billi. P., Barovic, G., Curovic, M., Sestras, P., and Khaledi Darvishan A. (2017b): Ecological-economic (eco-eco) modelling in the mountainous river basins: Impact of land cover changes on soil erosion. *Agriculture and Forestry,* 63 (4): 9-25.

West. J. (2019). Multi-criteria evolutionary algorithm optimization for horticulture crop management. *Agricultural Systems*. 173. 469-481. doi:10.1016/j.agsy.2019.03.016

World Customs Organization. 2018. What is the Harmonized System (HS)? [online]. In wcoomd.org. [cited 2018 – 03 - 20]. Available from Internet: http://www.wcoomd.org/en/topics/nomenclature/overview/what-is-the-harmonized-system.aspx

Wahab, R., Fauzi, M. N., Mokhtar, N., Sulaiman, S. M., Ghani, M. S. R., Edin, T. (2020): Enhancing mechanical properties of Rhizophora apiculata through engineered laminated boards. Agriculture and Forestry, 66 (3): 53-64.

DOI: 10.17707/AgricultForest.66.3.05

Razak WAHAB[1]*, Norashikin MOHD FAUZI[2], Nasihah MOKHTAR[2], Mohamad Saiful SULAIMAN[1], Ros Syazmini MOHD GHANI[1], Taharah EDIN[1]

ENHANCING MECHANICAL PROPERTIES OF *RHIZOPHORA APICULATA* THROUGH ENGINEERED LAMINATED BOARDS

SUMMARY

A study in enhancing the strength properties of *Rhizophora apiculata* by turning the solid wood into an engineered laminated board was executed. The matured *Rhizophora* species was selected from peat swamp forest in Sibu, Sarawak. The trunk was segregated into three parts, namely the bottom, middle and top portion and subsequently sawn into wooden planks. The laminated boards were cut into specific thicknesses and immediately bonded with an adhesive by pressed drying. Preparation for solid and laminated boards testing samples followed the ISO and ASTM standards. Properties such as the moisture content, density, basic density, static bending (MOR and MOE) and compression were determined. The results show an improvement in properties from the solid to laminated board. The values in density, MOR, and MOE increased in the range of 2-6%, 29-42%, and 13-36%, respectively. However, the values of the moisture content, basic density, and compression, indicated a vice versa trend ranging from 1-2%, 2-6%, and 8-24%, respectively. The bottom portion was more durable and robust compared to the other portions.

Keywords: *Rhizophora apiculata*, solid wood, engineered laminated boards, physical and strength properties.

INTRODUCTION

Peat swamp mangrove forests inhabited the coastal and riverine shores of the tropics and sub-tropics (Zhang et al., 2015; González 2012; Chandra et al., 2011) and constituted a dominant coastal vegetation community in tropical Asia where Malaysia-Indonesia is the centre of distribution (Abdullah et al., 2018). Seventy species of mangrove plants distributed worldwide and divided into 20 genera (Spalding et al., 2010). According to Duke (2006), *Rhizophora apiculata*

[1]Razak Wahab, (corresponding author: drrazakw5181@ucts.edu.my), Mohamad Saiful Sulaiman, Ros Syazmini Mohd Ghani, Taharah Edin, 1University College of Technology Sarawak (UCTS), 96000 Sibu, Sarawak, MALAYSIA

[2] Norashikin Mohd Fauzi, Nasihah Mokhtar, Universiti Malaysia Kelantan, 17699 Jeli Campus, Jeli, Kelantan, MALAYSIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:31/03/2020 *Accepted:01/06/2020*

is a species of plant in the *Rhizophoraceae* family. The main agroforestry uses from this species are soil stabilization, coastal protection, wildlife/marine habitat for marine fauna and also as timber products which are fuelwood, charcoal, dyes, and traditional medicines (Alongi 2008; Mazda et al., 2007). Nowadays, mangrove species can be further enhanced in constructions of dwellings, making furniture, rafts, boats, fences and even as a dying agent from the extracted tannin (Nabihah 2015).

Economically the mangroves forests consist of both monetary and non-monetary such as forest industry, fisheries industry, wildlife conservation, tourism and environment protection (Chandra et al., 2011; Bennet and Reynold 1993). The most typical representatives' species in the mangrove forest are the *Rhizophora apiculata*, *Rhizophora mucronata* and *Rhizophora mangle* (Halim et al., (2013). The mangrove trees is a complex combination of roots, trunk, branches and leaves (Zhang et al., (2015), the trees can grow up to 30 metres in height with trunk diameters up to 0.5 metres in width, elliptic-shaped leaves stained dark green with simple green in the middle and reddish-brown at the base of the leaves, bisexual flowers, round-shaped fruit up to pearl-shaped and brown, 18-38 cm long and 1-2 cm wide (Sugiarto 2019). On properties perception, wood density is a critical characteristic defining the mechanical properties of the wood and its performance with high wood density has been found to decrease vessel implosion by reducing the mechanical stresses associated with the negative pressure in the water column during drought (Santini et al., 2013). Greenwood of the mangrove *R. apiculata* trunk possesses higher density than water, but dry mangrove wood with a lower density floats in seawater and resistance to the marine deterioration (Zhang et al., (2015).

Wood and modified wood-based materials have long been used in many applications due to their excellent features in aesthetic appearance, reasonable cost, ease of use, low density, high mechanical strength, etc. (Percin et al., 2015). Excellent mechanical properties enable them to be utilized in a broad range of products especially after undergoing modification in the production of layered (laminated) wood with better strength or bending properties (Gáborík et al., 2016). Wood laminating materials are obtained by bonding two or more layers with adhesive and joining the fibre directions of the layers parallel or perpendicular to each other (Cibo et al., 2018). Laminating is a technique that improves the value of the wood material while enabling changes in the wood's properties and can be altered via in various ways which comprise multiple layers of wood, most frequently in the form of veneers (thin slices), glued and pressed together (Gáborík et al., 2016). The established laminated products which are oriented strand board (OSB), laminated veneer board (LVL), Strand-based composites include parallel strand board (PSL), laminated strand board (LSL) and oriented strand board (OSL), and also Glue-laminated timber (GLULAM) (Shmulsky and Jones 2019; Wiegner et al., 2009; Ong 2015). The focal point of this study was to determine the strength of physical and mechanical properties via solid wood and laminated wood. The differences properties between portions

(bottom, middle, and top) were also investigated. Previous studies related to cross-laminated bamboo, concentrated on the mechanical properties such as compressive, tensile, shear, and bending resistance no experimental procedure proposed for mangrove species, *Rhizophora apiculata*. More studies on mangrove species are needed by virtue of this study fills the identified research gap by focusing on its mechanical property's enhancement through engineered laminated boards.

MATERIAL AND METHODS

Sample Preparation

The *Rhizophora apiculata* used in this study was harvested from a mangrove forest in Sibu, Sarawak. The mangrove tree with an average height of 15 m and a diameter of 20 cm at dbh was selected. The tree was removed from the soil and cut into three (3) parts namely, the bottom, middle and top portion. The bottom, middle and top parts were part was cut 50%, 30% and 20% of the total length of the respectively. Subsequently, the samples was cut into the measurable sizes for physical and mechanical testing. The samples were divided into two groups namely solid-wood and laminated wood. The samples were cut by using a band saw into specific sizes and thickness. In group 2 (laminated), the samples with specific thickness were bonded together to perform as laminated wood. PVC glue branded, Pye Bond was used for the process. Samples were conditioning for 48 hours using clamping jig to maintain the position and imposed the pressure. The procedure followed the International Organization for Standardization, ISO 3131-1975 (Sulaiman et al., 2018; Wahab et al., 2017a) and the American Standard Testing Method, ASTM D 143 for mechanical testing (Ghani et al., 2018). The test was conducted according to the standard specification, International Organization for Standardization, Wood-determination of Density of Physical Test, ISO 3131-1975 (Sulaiman et al., 2018; Wahab et al., 2017a). The physical tests was carried out to determine the moisture content, density and basic density.

Moisture Content

The method that was used to determine the moisture content was based on oven-dry weight. In this study, the samples were cut into the following measurement of 25 mm x 25 mm x 25 mm with weight approximately 1–8g. All test was carried out according to the International Organization for Standardization which is ISO/TR 22157 (Wahab et al., 2018a; ISO / TR 22157 (2004).

Determination of Density and Basic Density

Density defined as the mass per unit volume, which is the moisture content of sample, at 12%. Basic density is defined as the mass per unit volume in oven-dry condition (Sulaiman et al., 2018; Wahab et al., 2018a). Samples of size 10 mm x 30mm x 30 mm were taken and oven-dried for 48 hours at 105±2°C to attain a constant weight. The samples were then weighed to obtain the oven-dried weight. To determine the green volume, the samples were placed into water under

a vacuum of about 700 mm Hg for 24 hours until thoroughly saturated. The volume of the fully saturated samples was then obtained using the water displacement method.

Mechanical Test

The mechanical test was executed based on the standard specifications, American Standard Testing Method, Standard Test Methods for Mechanical Properties of Wood-based Structural Material, ASTM D 143-2 (Wahab et al., 2018b; Khalid et al., 2015; Anonymous 1974) with modification. Two tests were carried out namely, static bending tests and compression tests in parallel with the grain. The mechanical properties of samples were measured by using universal testing machine (UTM Instron 3639). Prior to the mechanical testing, the labelled samples were stored under a standard temperature of 20 ± 2^{o}C and 65 ± 5% Relative Humidity (RH) or at least in a cold room and uniform temperature.

Statistical Analysis

Results presented as a mean ± standard error. The statistical analyses of experimental findings based on the one-way Analysis of Variance (ANOVA). A significant difference was statistically considered at the level of $P<0.05$. All experiment were triplicated (n=3).

RESULTS AND DISCUSSION

Investigate of Moisture Content

Three (3) types of physical tests conducted on mangrove samples prepared in two groups, namely group 1 (sample in solid-state) and group 2 (sample in double-layer laminate).

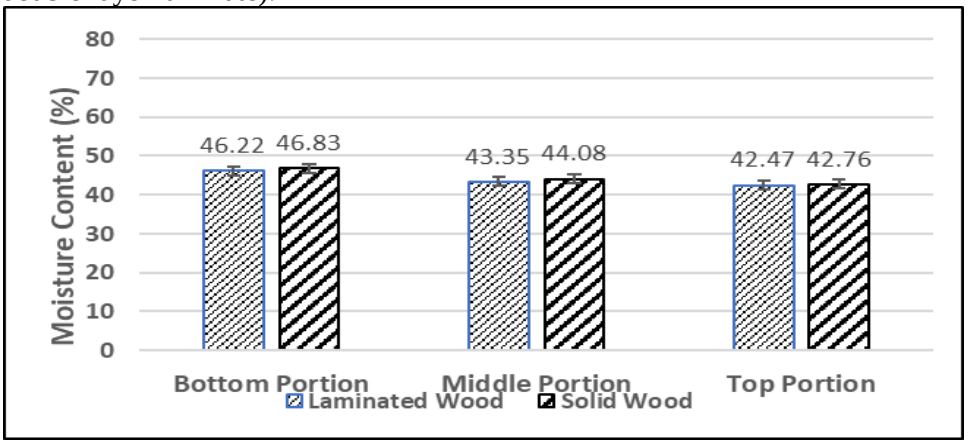


Figure 1: Moisture content for laminated wood and solid wood at different height portion

The measured physical properties comprised of moisture content (% MC), density (g/cm3) and basic density (g/cm3). Overall, the result for moisture content, indicated that the bottom sample recorded the highest moisture content (46.83%). The moisture content of the middle portion was recorded with 44.08%. The lowest percentage of moisture content was shown at the top portion with 42.761%. The graph of moisture content in sample group 2 (double-layer

laminate conditions) indicated a similar trend. The highest percentages of moisture content was recorded in the bottom portion with 46.221%, followed by the middle and top portion with 43.352% and 42.473%, respectively. The statistical analysis (Table 1) highlighted there was no significant differences between the portions (bottom, middle, and top) to both groups (solid and laminated).

That result was influenced by different between trees and species or in the same tree. On a single tree, glucose or wood core moisture content or parts of each tree may be different. This may be due to the moisture content of the tree is influenced by the age of the tree, species, external factors such as the condition or area of growth of the tree and internal factors such as the structure of the wooden anatomy. Mangrove tree *Rhizophora apiculata* is a kind of tree where the base of the tree is soaked in a state of stagnant water all year round. The base part of the root, including the root was the closest part to the soil and serves to facilitate the absorption of water to other parts of the tree. Based on the results obtained, the bottom of mangrove trees has the highest percentage of moisture content compared to other parts, due to the presence of water around the habitat. Moisture content significantly affects the mass of the wood, its dimensions, volume, physical, and mechanical properties (strength), and its resistance to attacks by fungi, molds, and insects (Wałach et al., 2015).

Differentiation of Density and Basic Density

Figures 2 and 3, the result in group 1 (solid) indicated the highest density at a bottom portion with 0.91 g/cm^3 and followed by the middle and top portion with 0.88 g/cm^3 and 0.86 g/cm^3, respectively. In group 2 (laminated), the density were recorded as 0.93 g/cm^3, 0.91 g/cm^3, and 0.83 g/cm^3 for the bottom, middle, and top, respectively. According to Table 1, statistical analysis shows there was not significantly different between the portions at group 1 (solid). There was significant differences between portions at group 2 (laminated) with value $p \leq 0.05$. Nevertheless, the graph indicated that the trend of basic density in group 1 (solid) decreasing from bottom to the top with 0.80 g/cm^3, 0.76 g/cm^3, and 0.705 g/cm^3. In group 2 (laminated), it indicated that the highest basic density at the bottom of 0.752 g/cm^3 followed by the middle and top portions with 0.74 g/cm^3 and of 0.68 g/cm^3, respectively. The statistical analysis (Table 1) shows there were significant differences between portion for both groups (solid group and laminated group) with value $p \leq 0.05$. The difference in density of wood in parts is influenced by the structure of the wood anatomy, size and arrangement of cells, length and density of fiber, cell wall thickness and chemical content in the wood (Desch 1989). In addition, the moisture content in wood cells can affect the value of wood density, the specific gravity of wood, wood dimensions and other wood strengths. High moisture content increases the density value (Thelandersson and Larsen 2002). Based on the results obtained from the physical properties test, it can be explained that the laminated wood has slightly higher in density compared to the solid wood.

Figure 2: Density for laminated wood and solid wood at different height portion

Figure 3: Basic density for laminated wood and solid wood at different height portions

Table 1: Data analysis for physical tests in group 1 (solid) and group 2 (laminated)

Group	**Tests Type**	**Sum of square**	**Mean square**	**P-Value**	**Sig. level**
Solid	MC	34.5386	17.2693	0.1448	ns
	Density	0.0057	0.00288	0.1465	ns
	Basic Density	0.0170	0.0085	0.0261	*
Laminated	MC	30.7368	15.3684	0.2981	ns
	Density	0.0229	0.0114	0.0188	*
	Basic Density	0.0110	0.0055	0.0124	*

*** : significant at P < 0.01; * : significant at P < 0.05; ns ; not significant*

Figure 4: MOR for static bending test on laminated wood and solid wood solid at different height portions.

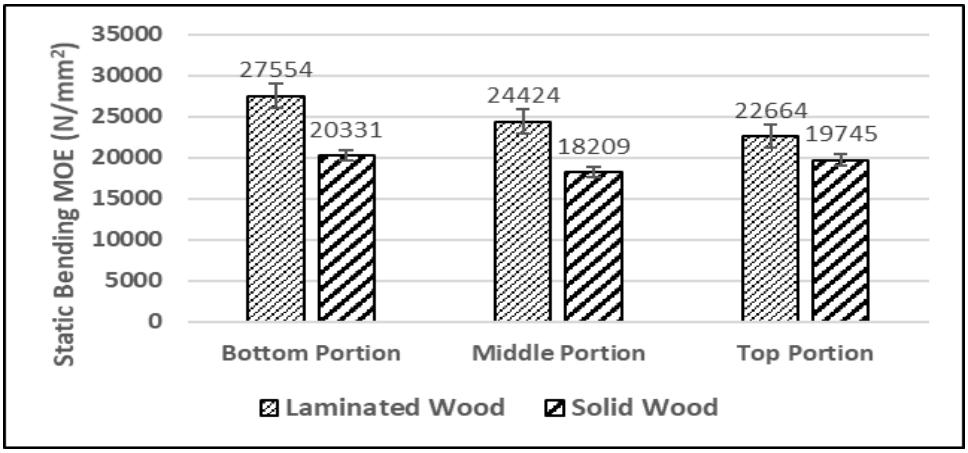


Figure 5: MOE for static bending of laminated wood and solid wood at different height portions.

The results supported by Percin et al., (2015), highlighted that density of the laminated wood materials has more superior values than the solid wood materials which were representing their kinds. The increase was assumed to be due to glue usage and layered structure.Determination of Modulus of Rupture (MOR) and Modulus of Elasticity (MOE) on Static Bending Test.

The result (Figure 4) in group 1 (solid) indicated that bottom portion has the highest MOR value with 168.200 N/mm^2 and followed by the top and middle portions which were167.875 N/mm^2 and 165.275 N/mm^2, respectively. Whereas in group 2 (laminated), it indicated the trend of MOR value decreasing from bottom to the top, with the value of 284.825 N/mm^2, 256.850 N/mm^2 and 237.350 N/mm^2, respectively. There was no significant difference between portions in group 1 (solid) (see Table 2). Notwithstanding, there was a significant difference

between portions in group 2 (laminated) with value of $p \leq 0.05$. Figure 5 indicated the MOE in group 1 (solid) has a higher value on the bottom portion with 20330.805 N/mm^2 and followed by the top and middle portion which is 19744.858 N/mm^2 and 18208.875 N/mm^2, respectively. Meanwhile in group 2 (laminated) highlighted the MOE value in decreasing order from bottom to the top, which is 27553.660 N/mm^2, 24424.423 N/mm^2, and 22664.093 N/mm^2, respectively. Nevertheless, (Table 2) the statistical analysis in group 1 (solid) and group 2 (laminated) highlighted there was no significant difference between a portion ($p \geq 0.05$).

Table 2: Table 1: Data analysis for static bending tests in group 1 (solid) and group 2 (laminated).

Group	Test	Sum of square	Mean square	P-Value	Sig. level
Solid	MOR	20.5617	10.2808	0.945	ns
	MOE	4.9065	2.45325	0.616	ns
	Compression	65.838	32.919	0.2301	ns
Laminated	MOR	4647.43	2323.72	0.0289	*
	MOE	9.60688	4.80344	0.155	ns
	Compression	472.178	236.089	0.0007	**

*** : significant at P < 0.01; * : significant at P < 0.05; ns ; not significant*

The factor that influence the evaluation of wood strength is density. The mechanical characteristics or wood strength, such as static wood bending is closely related to the density of the wood (Rasat et al., 2011; Haygreen and Bowyer 1930). The static flexural strength of the wood will increase by increasing the density from the base to the top. It can be explained that the base portion of mangrove trees Rhizophora apiculata has the highest static bending test value also has the highest density value. The strength of wood is also influenced by other factors such as genetics, tree parts, presence of timber knots, wooden anatomy structures, length and density of cells, tree age or species (Desch 1989; Khalid et al., 2010).

Determination of Compression Test

Figure 6 shows the highest value of compression on group 1 (solid) at a bottom portion with 71.593 N/mm^2 and followed by the top and middle portion with respectively around 68.715 N/mm^2 and 65.855 N/mm^2. The result in group 2 (laminated) indicated that the compression value decreasing from bottom to the top portion with 65.955 N/mm^2, 64.653 N/mm^2, and 52.045 N/mm^2. The statistical analysis result (Table 2) highlighted that there was no significant differences between portions in group 1 (solid) whereas group 2 (laminated) indicated that there was a significant differences between portions with value $p \leq 0.01$.

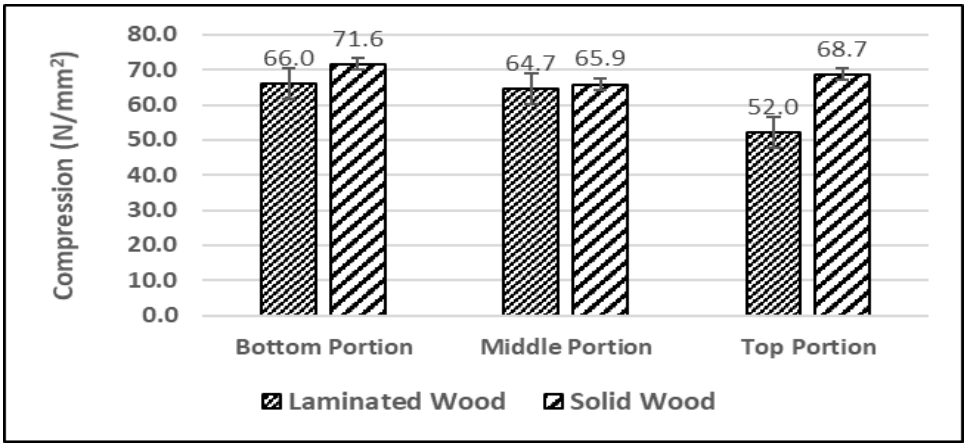


Figure 6: Compression test on solid wood and laminated wood at different height portions.

High density is an important factor that influences the results. Mechanical characteristics or wood strength such as wood compression strength are closely related to the density of wood in which the density of wood is an important feature in determining the strength of a tree (Wahab et al., 2017b; Haygreen and Bowyer 1930). The compressive strength of the wood will increase with increasing density from the base to the top. Thus, it can be explained that the apiculata mangrove root base has the highest compression test value also has the highest density value. However, the strength of wood is also influenced by internal factors such as genetics, tree parts, presence of timber book, wood anatomy structure, length and density of cells, tree age or species and external factors such as growth areas (Desch (1989).

CONCLUSIONS

Results of the study on different properties of matured *Rhizophora apiculata* indicated an improvement in the physical and mechanical properties from solid to the engineered laminated wood. In the physical properties, the moisture content highlighted that the decreasing order from bottom to the top and from solid wood to the laminated wood in the range 1-2%. Density indicated an increase in values from solid to the laminated wood with a variety of around 2-5% while the basic density shows the vice versa results in a variation of 2-6%.

In the mechanical properties, the static bending highlighted the MOR and MOE value improved the strength from solid to the laminated by the range 29-42% and 13-26%, respectively. The compression strength, however, shows the solid wood are stronger compared than laminated wood with a range of around 8-24%.

The study also found that the bottom portion indicated the highest value in physical and mechanical testing.

The factor that influenced the results may be due to the natural morphological in *R. apiculata* with content, high fibre strength and also complex microstructure at the bottom portion.

ACKNOWLEDGEMENTS

This study was financed by the University College of Technology Sarawak Grant (UCTS/RESEARCH /4/2017/01). The authors expressed their gratitude to University College of Technology Sarawak (UCTS) and Forest Research Institute Malaysia (FRIM) for permission on using their laboratory and workshop equipment in the preparation and analysis parts of the study.

REFERENCES

Abdullah F, Shaari H, Satyanarayana B, Khalik WMAWM, Jaafar MZM (2018). Macro, Micro, and Non-Essential Elements in Different Parts of *Rhizophora Apiculate. Malaysian Journal of Analytical* Sciences, 22(4): 570 – 578. DOI: 10.17576/mjas-2018-2204-01.

Alongi DM (2008). Mangrove forests: resilience, protection from tsunamis, and responses to global climate change. Estuarine, Coastal and Shelf Science, 76(1), 1-13.

Anonymous (1974). *The Strength Properties of Timber*. MTP Construction. England: Medical and Technical Publishing Co. Ltd.

Bennet EL and Reynold CJ (1993).The value of a mangrove area in sarawak. Biodiversity Conservation, 2: 359-375. DOI:10.1007/BF00114040.

Chandra IA, Seca G, and Abu Hena MK (2011). Aboveground Biomass Production of *Rhizophora apiculate* Blume in Sarawak Mangrove Forest. American Journal of Agricultural and Biological Sciences 6 (4): 469-474.

Cibo C, Altay C, Özçifçi A, Baysal E, Toker H (2018). Determination of Some Mechanical Properties of Laminated Wood Material Reinforced with Carbon Fiber (CFRP). Mesleki Bilimler Dergisi (MBD), 7 (2): 123 – 132.

Desch HE (1989). *Struktur, Sifat dan Penggunaan*. Terjermah oleh Mohd. Zin Jusoh and Mohd. Hamami Sahri. Edisi ke enam. United Kingdom: The Macmillan Press.

Duke NC (2006). *Rhizophora apiculata, R. mucronata, R. stylosa, R. X Annamalai, R. X lamarckii* (Indo-West Pacific stilt mangrove).

Gáborík J, Gaff M, Ruman D, Záborský V, Kašíčková V, Sikora A (2016). Adhesive as a Factor Affecting the Properties of Laminated Wood. Adhesive and lamination, *BioResources* 11(4), 10565-10574. DOI: 10.15376/biores.11.4.10565-10574.

Ghani RSM, Wahab R, Azmi SNB, Wi KM, Mokhtar N, Sulaiman MS (2018a). Comparison of Properties between Solid and Laminated Mahang Wood. *Advanced Journal of Technical and Vocational Education*, 2(2): 24-28.

González NSS (2012). Structure and function of wood in mangroves. Thesis in Doctor of Philosophy at the University of Queensland.

Halim NHA, Abidin NAZ, Me R (2013). A Study of Chemical Compounds in *Rhizophora apiculata*. *The Open Conference Proceedings Journal*, 2013*(4)*, (Suppl-2, M24) 108-110.

Haygreen JG, Bowyer JL (1930). Introduction to Forest Product and Wood Science, Subtitled by Suhaimi Muhammed and Sheikh Abdul Karim Yamani Zakaria. Ampang Press Sdn. Bhd., Kuala Lumpur.

ISO / TR 22157 (2004). Bamboo: Determination of Physical and Mechanical Properties.

Khalid I, Sulaiman O, Hashim R, Wahab R, Jumhuri N, Rasat MSM (2015). Evaluation on Layering Effects and Adhesive Rates of Laminated Compressed Composite Panels made from Oil Palm (*Elaeis guineesis*). Journal of Materials and Design. 68: 24-28. DOI:10.1016 / j.matdes. 2014.12.007, Elsevier Science Direct UK.

Khalid I, Wahab R, Mahmud S, Othman S, Affendy H, Hanim RA & Andy RM (2010). Chemical Changes in 15-year-old Cultivated *Acacia hybrid* Oil-Heat Treated at 180, 200 and 220°C. International Journal of Chemistry 2 (1). Pp. 97-107. Feb. 2010. ISSN: 1916-9701. Canadian Center of Science and Education.

Mazda Y, Wolanski E, Ridd PV (2007). The Role of Physical Processes in Mangrove Environments: Manual for the Preservation and Utilization of Mangrove Ecosystems. Terrapub, Tokyo, 598 pp.

Nabihah A (2015). Bioactivities of Methanol Extracts from *Rhizophora apiculata* Blume and *Rhizophora mucronata* Lam. Barks. BSc. Thesis. Universiti Malaysia Sarawak (UNIMAS).

Ong CB (2015). Glue-laminated timber (Glulam). In Wood Composites (pp. 123-140). Woodhead Publishing.

Percin O, Sofuoglu SD, Altunok M (2015). Effect of Heat Treatment on Some Physical and Mechanical Properties of Laminated Wood. The XXVII International Conference Research for Furniture Industry, Ankara, Turkey.

Rasat MSM, Wahab R, Sulaiman O, Janshah M, Aminuddin M, Tamer AT, Izyan K (2011). Properties of bio-composite board from oil palm frond agricultural waste. Bioresources Journal 6(4): 4389-4403. Sep. 2011.

Santini NS, Schmitz N, Bennion V, Lovelock CE (2013).The anatomical basis of the link between density and mechanical strength in mangrove branches. *Functional Plant Biology*, 2013, 40, 400–408. doi.org/10.1071/FP12204.

Shmulsky R, Jones PD (2019). Forest products and wood science: an introduction. John Wiley & Sons.

Spalding M, Kainuma M, Collins L (2010). World atlas of mangroves. Earthscan, London.

Sugiarto A (2019). Supplemental materials for preprint: Pertumbuhan Akar Propagul Rhizophora apiculate Blume Pada Medium Air Tawar. Retrieved from osf.io/krpa.

Sulaiman MS, Ramle SFM, Hashim R, Sulaiman O, Amini MHM, Geng BJ (2018). The classical mechanics engineered of *Bambusa vulgaris* and *Schizostachyum brachycladum.* Journal of Tropical Resources and Sustainable Science 6:57-61.

Thelandersson S, Larsen HJ (2002). *Timber Engineering*. John Wiley & Sons, Ltd.

Wahab R, Ghani RSM, Samsi HW & Rasat MSM (2017b). Changes in the Features of Oil Heat Treated 18-Years Old *Acacia mangium*. Research Journal of Pharmaceutical, Biological and Chemical Sciences 8 (2): 2093-2106. ISSN: 0975-8585.

Wahab R, Mokhtar N, Ghani RSM, Sulaiman MS, Samsi HW (2018a). Changes in Strength Characteristics and Durability on 4-Year-Old Tropical Bamboo *Gigantochloa Scortechinii* through Heat Treatment. *Asian Journal of Science and Technology, 9(5):8227-8233.*

Wahab R, Rasat MSM, Samsi HW, Mustafa MT, Don SMM (2017a). Assessing the suitability of Agro-Waste from Oil Palm Empty Fruit Bunches as Quality Eco-Composite Boards. Journal of Agricultural Science 9 (8): 237-247. ISSN 1916-9752 E-ISSN 1916-9760. Doi: 10.5539/jas. v9n6p251. Published by Canadian Center of Science and Education.

Wahab R, Sulaiman MS, Mokhtar N, Ghani RSM, Samsi HW, Yusuf M (2018b). Properties of *Hibiscus cannabinus* and *Elaeis guineesis* fronds fibers Eco-

Composites boards. International Journal of Current Research 10 (5): 69770-69775. ISSN: 0975-833X.

Wałach D, Jaskowska-Lemańska J, Dybeł P (2015). The impact of the moisture content of wood on the results of non-destructive tests. Forestry and Wood Technology № 92: 448-454.

Wiegner K, Wilke O, Jann O, Brödner D, Scheffer H, Kalus S, Till C (2009). Study on VOC-emissions from oriented strand boards (OSB). In Proceedings of the 9th International conference Healthy Buildings.

Zhang X, Chua VP, Cheong HT (2015). Hydrodynamics in mangrove prop roots and their physical properties. Journal of hydro-environment research. 9(2):281-294. DOI: 10.1016/j.jher. 2014.07.010.

Maksimović, T., Janjić, N., Lubarda, B. (2020): Effect of different concentrations of mannitol on germination of pea seeds (Pisum sativum L.). Agriculture and Forestry, 66 (3): 65-72.

DOI: 10.17707/AgricultForest.66.3.06

Tanja MAKSIMOVIĆ, Nina JANJIĆ, Biljana LUBARDA [1]

EFFECT OF DIFFERENT CONCENTRATIONS OF MANNITOL ON GERMINATION OF PEA SEEDS (*Pisum sativum* L.)

SUMMARY

During the life cycle, plants are exposed to various abiotic and biotic stress factors that adversely affect their growth, development and productivity. Drought is one of the stress factors affecting the decline in plant growth and productivity worldwide. In this study, the effect of water deficiency caused by different concentrations of mannitol (5%, 10%, and 20%) on the germination of seeds of two varieties of peas (Petit Provencal and Joff) was studied. The results of the study showed that the germination percentage and germination potential of both pea varieties decreased with increasing mannitol concentration compared to the control. The Petit Provencal variety had a higher percentage of germination, germination potential and vigor index of drought resistance and this variety was considered more tolerant to drought stress.

Keywords: drought, mannitol, seed, germination, *Pisum sativum* L.

INTRODUCTION

In the past few years, drought has been one of the most common factors damaging plant growth and development, and is becoming an increasingly serious problem in many regions around the world. Extreme water shortages cause considerable physiological, metabolic and morphological changes in plants which in turn reduce the yield and quality of the crop (Al-Jebory, 2012; Duan et al., 2017; Chaves and Oliveira, 2004).

Pea (*Pisum sativum*) is a very important agricultural crop from the legume (Fabaceae) family that plays a large role in human nutrition. It is of particular importance primarily because of its high nutritional value, intensive production and its short cultivation period (Kumar and Choundhary, 2014). Since peas are grown in climatically different regions under different production conditions, pea seeds are inevitably susceptible to various stress conditions. Stressful conditions inhibit seed germination, resulting in poor crops, reduced biomass, and ultimately, reduced yield and quality (Machado Neto et al., 2004;

[1]Tanja Maksimović, (corresponding author: tanja.maksimovic@pmf.unibl.org), Nina Janjić, Biljana Lubarda, Department of Biology, Faculty of Natural Sciences and Mathematics, University of Banja Luka, Mladena Stojanovića 2, 78 000 Banja Luka, Republic of Srpska, BOSNIA AND HERZEGOVINA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:02/06/2020 *Accepted:08/07/2020*

Khodarahmpour, 2011; Al-Jebory, 2012; Duan et al., 2017). Water stress acts by reducing the percentage, germination rate and seed growth. The adverse impact of water scarcity has been well studied in different crops such as maize (Radić et al., 2007; Khodarahmpour, 2011; Jain et al., 2013; Liu et al., 2015), soybean (Machado Neto et al., 2004), peas (Al-Jebory, 2012), wheat (Koka et al., 2015; Duan et al., 2017), and sunflower (Luan et al., 2014).

The most popular technique for causing drought is by using high molecular weight substances such as mannitol and polyethylene glycol. Mannitol is a white, crystalline solid of the chemical formula $C_6H_8(OH)_6$ which does not pass through the cell wall, has low toxicity so it is an ideal material used to simulate arid soil (Al-Jebory, 2012; Liu et al., 2015).

The aim of this paper is to study the differences in germination and seed growth of drought-exposed seeds in two varieties of peas to determine which variety is more tolerant and suitable for cultivation in water scarcity conditions.

MATERIAL AND METHODS

In this paper, the effect of water deficit on seed germination and seedling growth in two varieties of peas, Petit Provencal and Joff, was monitored. The pea seeds were first sterilized with 70% ethanol and then washed with distilled water. Each variety was germinated in sterile petri dishes containing 30 seeds in 3 repetitions. The seeds were treated with different concentrations of mannitol from the beginning: 5%, 10% and 20%, and distilled water was used in the control. After setting up the experiment, seed germination was performed at a temperature of 26°C in a thermostat. The percentage of germination was monitored on the third, fifth, and seventh days, and after seven days, the germination potential, vigor index of drought resistance, and seedlings growth were calculated.

Germination percentage

Germination percentage was calculated as follows:

Germination percentage= (germinated seed number/total seed number) x 100% (Liu et al., 2015).

Germination potential

Germination potential is an index that shows the relationship between the percentage of germination and the uniformity of germination, and it is calculated as follows:

Germination potential= (germinated seed number at germination peak/total seed number) x 100% (Liu et al., 2015).

Germination and vigor index of drought resistance

Germination and vigor index of drought resistance were calculated as follows: drought resistance index of germinated seed = (seed promptness index under water deficiency- PIS)/ seed germination promptness index -PIC (control); Promptness index PI= $(nd_2 \cdot 1.00) + (nd_4 \cdot 0.75) + (nd_6 \cdot 0.50) + (nd_8 \cdot 0.25)$; where nd_x= number of germinated seeds by the xth day of measurement (Grzesiak et al., 2013).

After seven days, the growth of seedlings and roots was measured in control and treated seeds.

All results are presented as the average value of the three replicas ± standard deviation (SD). The statistical significance of the analyzed parameters was tested by ANOVA test ($p<0.05$) two-way analysis of variance.

RESULTS AND DISCUSSION

The seeds of the Petit Provencal variety germinated better than the Joff variety under different mannitol treatments. The percentage of germination in both pea varieties tested decreased with increasing mannitol concentration compated to the control (Figure 1).

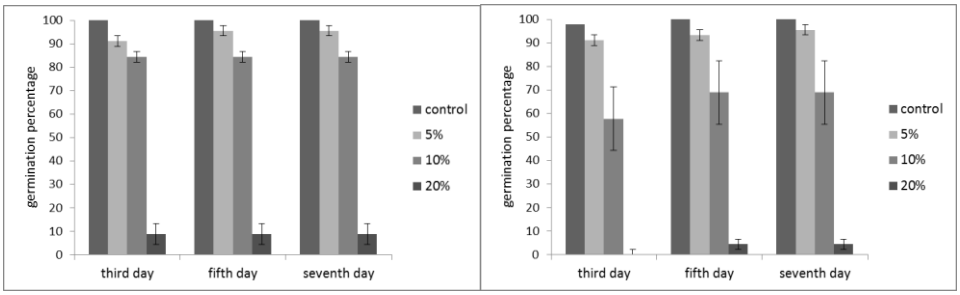


Figure 1. Germination percentage of seeds of two studied pea varieties (Petit Provencal - left, Joff- right) under different concentrations of mannitol

Under the influence of 5% mannitol solution, the percentage of seed germination of the Petit Provencal variety decreased by 4.5%; under the influence of 10% mannitol by 15.6%, and under the influence of 20% mannitol by 91.2% compared to the control. Under the influence of 5% mannitol, the germination percentage of the Joff variety was lower by 4.5% compared to the control, while under the influence of 10% it decreased by 31.1% and under the influence of 20% mannitol by 95.6%. Based on the results of the ANOVA test, it was concluded that there are no statistically significant differences between the same treatment depending on the day for both investigated varieties. Also, a statistically significant difference was obtained between different groups depending on the concentration of mannitol, both for the variety Petit Provencal and for Joff (Figure 1).

The germination potential of the two pea varieties decreased significantly due to mannitol treatment with significant differences between them. Under the influence of 5% mannitol, the potential of the Petit Provencal variety decreased by 33.3% and in the Joff variety by 46.7%. Under the influence of 10% mannitol, the germination potential of the Petit Provencal variety decreased by 66.6%, while in the Joff variety it decreased by 80%. At the highest concentration of mannitol, no germination of seeds of any variety occurred. The results of the ANOVA test for the germination potential parameter show that there are statistically significant differences between different varieties at a mannitol

concentration of 5% and 10%. Statistically significant differences also exist between different concentrations of mannitol in the same pea variety (Figure 2).

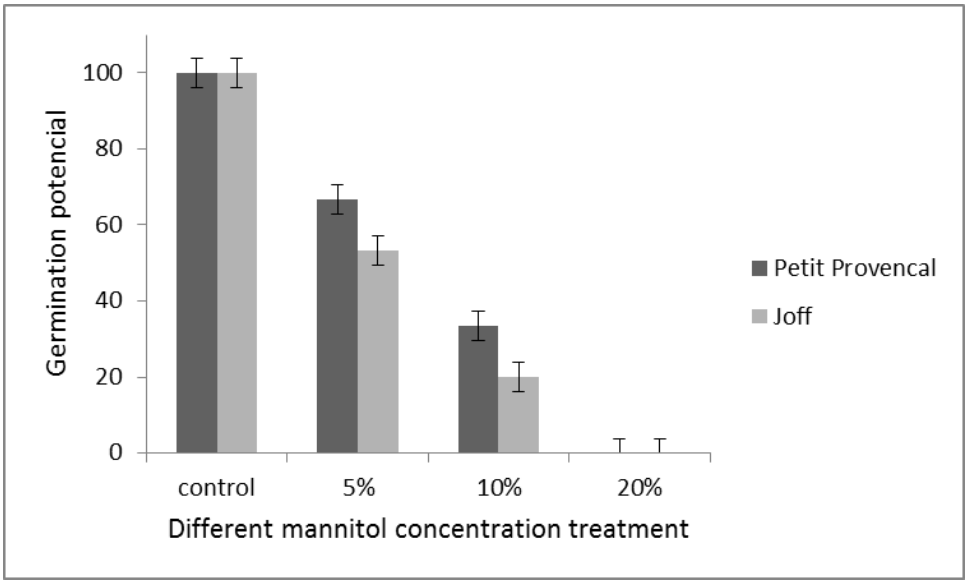


Figure 2: Germination potential of two studied pea varieties under different concentrations of mannitol

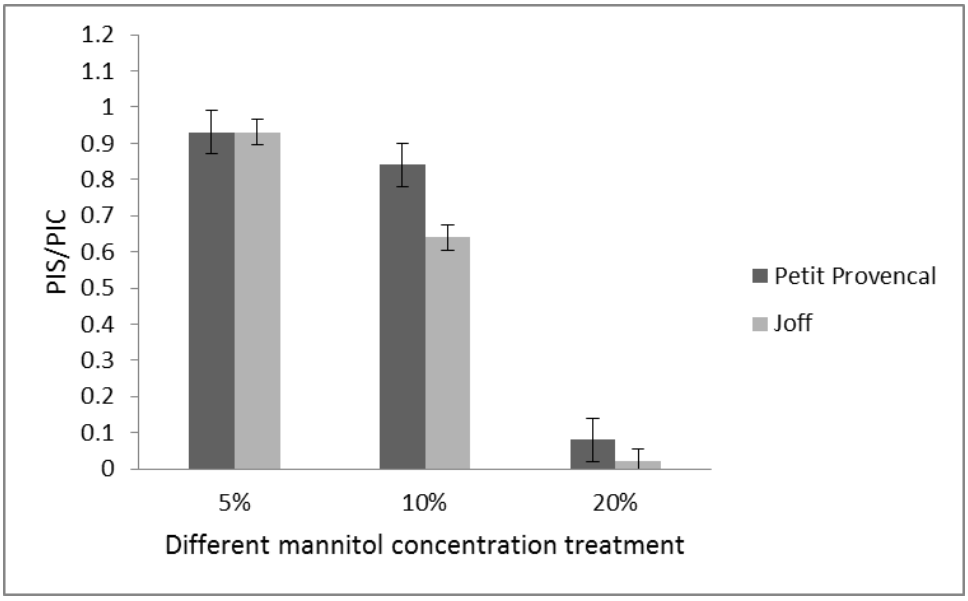


Figure 3: Vigor index of drought resistance in studied pea varieties

Vigor index of drought resistance (PIS/PIC) of the two pea varieties decreased with increasing mannitol concentration. In the case of the Joff variety

PIS/PIC with the treatment of 10% and 20% of mannitol decreased more than in the Petit Provencal variety, while under the influence of 5% of mannitol the value of this parameter was uniform in both varieties. As for the germination potential parameter, the test shows that for the vigor index there are statistically significant differences between different varieties at a mannitol concentration of 10% and 20%. As for the germination potential parameter, the test shows that for the vigor index there are statistically significant differences between different varieties at a mannitol concentration of 10% and 20% (Figure 3).

The average root length of the Petit Provencal variety was greater than the Joff variety and decreased on average by 2-4 times under the influence of mannitol compared to the control (Figure 4). The highest root length was recorded in control. The mean value of the root length of the Petit Provencal variety under the influence of 5% mannitol decreased by 75.7%, and under the influence of 10% mannitol by 77.3%. In the Joff variety under the influence of 5% mannitol, the root length decreased by 50.9%, and under treatment with 10% mannitol by 60.4% compared to the control. These values were measured on the seventh day. There is a statistically significant difference in root length depending on the concentration of mannitol, and also between the fifth and seventh day for the same variety. In addition, there is a statistically significant difference between different varieties in the same treatments especially for a concentration of 10%. No root was present in the treatment with 20% mannitol in both varieties of peas.

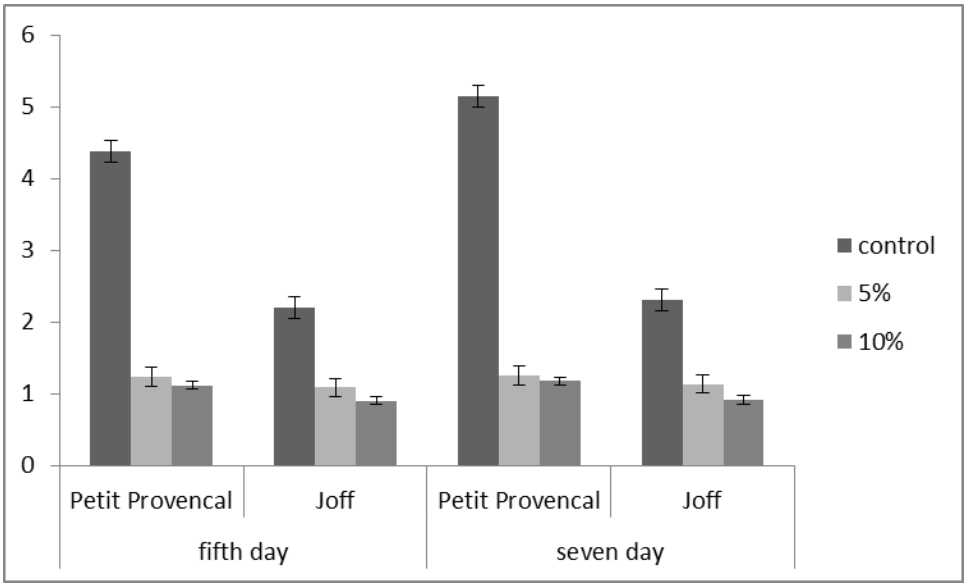


Figure 4: Average root length [cm] in Petit Provencal and Joff variety

No seedling was present in both pea varieties, indicating that administration of mannitol concentration (5%, 10%, and 20%) completely inhibited plant growth.

Plants are exposed to various stress factors throughout their life cycle that greatly affect their growth, development and productivity. Drought is among the first abiotic stressors, which limit the normal functioning of the plant (Machado Neto et al., 2004; Radić et al., 2007; Khodarahmpour, 2011; Al-Jebory, 2012; Jain et al., 2013; Luan et al. al., 2014; Liu et al., 2015; Koka et al., 2015; Duan et al., 2017). The effect of drought, on the level of the whole pea plant is multiple, and is reflected in the reduced ability to germinate and sprout seeds, poorer root development and above-ground part of the plant, reduced ability to create and accumulate dry matter, as well as a negative impact on flower formation, pollen formation, pollination, grain formation and quality (Al-Jebory, 2012; Elkoca, 2014).

In this paper, drought resistance of two varieties of peas under simulated mannitol-induced conditions was monitored. The percentage of germination of both varieties of peas declined under the influence of drought. By comparison of the tested varieties it was found that the percentage of germination was higher in the Petit Provencal variety compared to Joff. Treatment with the highest concentration of mannitol (20%) caused a 91.2% decrease in germination rates for the Petit Provencal variety and 96.5% for the Joff variety, indicating that the Petit Provencal variety exhibited higher drought resistance (Figures 1). These results agree with those of Machado Neto et al. (2004) for soybean and with Koka et al. (2015) for the oat.

According to Elkoca (2014) water uptake is directly related to the osmotic potential of the applied polyethylene glycol and mannitol in the experiment. More specifically, when treating seeds with these substances, the promptness and percentage of seed germination are reduced.

Similar studies were carried out by Machado Neto et al. (2004) on certain soybean varieties, whereby they found that the percentage of germination decreased depending on the concentrations of mannitol applied, which may be related to the results of our studies.

Germination potential is a parameter that shows the degree of germination and evenness of seed germination (Liu et al., 2015). In this paper, it was shown that the germination potential of both pea varieties declined under the influence of drought, but that it decreased more in the Joff variety than in the Petit Provencal variety (Figure 2), indicating that the Petit Provencal seed germinated faster than the Joff variety seed, showing at the same time stronger drought resistance. Water stress not only affects seed germination but also extends the period required for germination (Khodarahmpour, 2011; Liu et al., 2015), as we have noted in this paper.

The drought resistance index is a parameter that better and more accurately indicates to us the drought resistance and germination characteristics (Grzesiak et al., 2012; 2013). The resistance index for both tested pea varieties decreased with increasing mannitol concentration, but for the Petit Provencal variety decreased less than for Joff, which tells us that the Little Provencal variety showed greater resistance to drought (Figure 3). Liu et al. (2015) in their study on two maize

varieties also found a decrease in drought resistance index with increasing mannitol concentration.

From the obtained results, it was found that the Petit Provencal variety had longer roots under the influence of drought (Figure 4), implying that this variety had a more developed root system and therefore showed higher resistance than the Joff variety. Reduced root growth in 4 mannitol-induced soybean cultivars was reported by Machado Neto et al. (2004) as well as Koka et al. (2015) on the oat. Duan et al. (2017) reported in their study on wheat that inhibitions of root growth under the influence of polyethylene glycol-induced drought occur, and this may be related to the results obtained in this work. Khodarahmpour (2011) by studying 7 maize hybrids under drought stress conditions showed that root length decreased by up to 60% compared to the control.

The Petit Provencal cultivar had a higher percentage of germination and higher promptness, root length, indicating that this variety was more drought tolerant than the Joff variety.

CONCLUSIONS

The growth of plants in arid and semi-arid conditions depends on the susceptibility of the plants to drought and the ability of the seed to achieve optimal germination in adverse conditions. It is therefore of great importance to determine the tolerance of varieties to drought in the initial stages of growth. Taking all the characteristics into account, in this paper the Petit Provencal variety germinated faster and had a higher percentage and potential of germination than the Joff variety. Also, the cultivar Little Provencal had a better root system, which indicates a better ability for osmotic adaptation, which allows cultivation of the tested variety in dry conditions. This type of research shows that monitoring seed germination index can be used to determine the tolerance and sensitivity of a variety to the stress caused by drought in the initial stages of growth.

ACKNOWLEDGEMENTS

The authors acknowledge financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2020-14/ 200358; 200032) and DRAGON GA 810775 (Data Driven Precision Agriculture Services and Skill Acquisition), H2020-WIDESPREAD-05-2017-Twinning; and bilateral project (MNO & Serbia; 2019-2020): Alternative cereals and oil crops as a source of healthcare food and an important raw material for the production of biofuel; FAO Project (2019-2022): Redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain and improved farmers' livelihoods in Serbia and Bulgaria – GRAINeFIT.

REFERENCES

Al-Jebory E I. 2012. Effect of water stress on carbohydrate metabolism during Pisum sativum seedlings growth. Euphrates Journal of Agriculture Science, 4 (4); 1-12.

Chaves M M, Oliveira M M. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot., 55; 2365–2384.

Duan H, Zhu Y, Li J, Wang H, Zhou Y. 2017. Effects of Drought on Growth and Development of Wheat Seedlings. International Journal of Agriculture & Biology. 19(5);1119-1124. doi:10.17957/IJAB/15.0 393.

Elkoca, E. 2014: Osmo- and hydropriming enhance germination rate and reduce thermal time requirement of pea (*Pisum sativum* L. cv. Winner) seeds. Akademik Ziraat Dergisi, 3(1);1-12.

Grzesiak M T, Marcinska I, Janowiak F, Rzepka A, Hura T. 2012. The relationship between seedling growth and grain yield under drought conditions in maize and triticale genotypes. Acta Physiol Plantarum, 34; 1757-1764.

Grzesiak M T, Waligorski P, Janowiak F, Marcinska I, Hura K, Szczyrek P, Głab T. 2013. The relations between drought susceptibility index based on grain yield (DSIGY) and key physiological seedling traits in maize and triticale genotypes. Acta Physiol Plant, *35*; 549-565. http://dx.doi.org/10.1007/s11738-012-1097-5.

Jain M, Mittal M, Gadre R. 2013. Effect of PEG-6000 Imposed Water Deficit on Chlorophyll Metabolism in Maize Leaves. Journal of Stress Physiology. 9(3): 262-271.

Khodarahmpour, Z. 2011. Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.). African Journal of Biotehnolog, 10 (79); 18222-18227.

Koka J A, Wani A H, Agarwal R M, Parveen S, Wani F A. 2015. Effect of Polyethylene glycol 6000, mannitol, sodium and potassium salts on the growth and biochemical characteristics of oat (*Avena sativa* L.). European Academic Research, 3 (1); 303-314.

Kumar,A, Choundhary A K. 2014. Scientific Cultivation of Vegetable Pea (*Pisum sativum* L.). In book: Advances in Vegetable Agronomy. Publisher: Indian Agricultural Research Institute.

Liu, M, Li M, Liu K, Sui N. 2015. Effect of Drought Stress on Seed Germination and Seedling Growth of Different Maize Varieties. Journal of Agricultural Science, 7 (5); 231-240.

Luan Z, Xiao M, Daowei D, Zhang H, Tian Y, Wu Yi, Guan B, Song Y. 2014. Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (*Helianthus annuus* L.). The Scientific World Journal, 2014; 1-9.

Machado Neto B N, Saturnino S M, Bomfim D C, Custódio C C. 2004. Water Stress Induced by Mannitol and Sosium Chloride in Soybean Cultivars. Brazilian Archives of Biology and Technology, 47 (4); 521-529.

Radić V, Beatović D, Mrđa J. 2007. Salt tolerance of corn genotypes (Zea *mays* L.) during germination and later growth. Journal of Agricultural Sciences, 52 (2); 115-120.

Govedarica-Lučić, A., Perković, G., Rahimić, A., Bošković, I., Pašić, S. (2020): Influence of fertilization on growth and qualty of lettuce. Agriculture and Forestry, 66 (3): 73-80

DOI: 10.17707/AgricultForest.66.3.07

Aleksandra GOVEDARICA-LUČIĆ, Goran PERKOVIĆ[1], Alma RAHIMIĆ[2], Ivana BOŠKOVIĆ[1], Sanid PAŠIĆ[3]

INFLUENCE OF FERTILIZATION ON GROWTH AND QUALTY OF LETTUCE

SUMMARY

The biological characteristics of lettuce and its specific growth and development are the basis for establishing the optimal method of cultivation. In order to achieve appropriate high yields, producers various organic, mineral and microbial fertilizers.

The aim of the study was to determine the effect of fertilization on the growth and quality of different varieties of lettuce. A two-factor experiment (fertilization and variety) was set up on a random block system in a greenhouse without heating, on the territory of East Sarajevo. A non-fertilized control variant was included in the trial.

During the research, the effect of fertilizers (Slavol and Fitofert humisuper) on two varieties of lettuce (Santoro RZ and Kiribati RZ) was examined.

The highest mass of lettuce was recorded on the variant with application of Slavol (309,19 g).The highest percentage of dry matter was achieved by fertilization with Slavol in the Kiribati *RZ* variety – 6,46%, while the lowest percentage was achieved on the control variant in the Santoro RZ variety - 5.10%.

Keywords: fertilization, lettuce, yield, quality.

INTRODUCTION

Vegetable crops have high nutrient requirements. Proper growth and development of plants requires a ratio of nutrients found in the soil. Nutrition of plants, ie. the addition of the necessary nutrients to the plant is done by applying fertilizer. Today, organic and mineral fertilizers are used to feed the plants (Rašević, 2017). Organic fertilizers play a major role in the growth and development of plants. They contain the necessary macro and micro elements and

[1]Aleksandra Govedarica-Lučić, (corresponding author: a.govedaricalucic@pof.ues.rs.ba), Goran Perković, Ivana Bošković, Faculty of Agriculture, University of East Sarajevo, BOSNIA AND HERZEGOVINA.

[2]Alma Rahimić, Agromediterranean Faculty, University of "Džemal Bijedić", Mostar, BOSNIA AND HERZEGOVINA.

[3]Sanid Pašić, Federal Institute of Agriculture, Butmirska 18, BOSNIA AND HERZEGOVINA.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Received:08/04/2020 *Accepted:03/07/2020*

improve the physical and chemical properties of the soil (Chaterjee et al.,2005; Chaterjee et al.,2014; Čabilovski et al., 2010).

However, according to many researchers, fertilizing the soil with organic fertilizer for vegetable nutrition alone does not produce good results in achieving high and stable yields. Therefore, various types of organic fertilizers should be applied in combination with mineral fertilizers (Ogbonna, 2008; Ndaeyo et al., 2005; Makinde et al., 2007; Dauda et al., 2008). Mineral fertilizers are products containing nutrients essential for the normal growth and development of plants.As for the production of lettuce, it has been proven that organic fertilizers are more suitable than inorganic fertilizer (Masarirambi et al. 2010). Also, mineral fertilizers can have a detrimental effect on plant quality, declining dry matter content, increasing soil acidity, increases the nitrate concentration in the lettuce cultivar ,degeneration of physical properties, increasing erosion and instability of soil aggregates (Adeoluwa and Adeogun, 2010; Olowoake and Adeoye, 2010; Premuzic et al. 2001).

The application of microbiological fertilizers has economic and environmental justification. Fertilizers and lately bio stimulants have increasingly been used as a tool with the potential to enable a more sustainable agriculture production (Bulgari et al. 2015). Improvement of plant production goes towards significant use of microbial inoculants in the production of non-leguminous plants, including the most important crop and vegetable species. Because the aim of this study was to examine the effects of Slavol bio stimulant and the Fitofert fertilizer on production of the lettuce and draw a conclusion about the effectiveness of their action.

MATERIAL AND METHODS

The impact of different fertilization variants on the growth and quality of two lettuce varieties was investigated during one growing season in 2019. A two-factor experiment was set up in a greenhouse without heating. The experiment was set up according to a random block system, in three repetitions with the size of a test plot of 2 m² (1x2 m).

Within the first tested factor fertilization (A) were variants:a_1) control (no fertilization), a_2) Slavol, and a_3) Fitofert humisuper plus.

Slavol is a universal certified fertilizer and can be used in organic and traditional agricultural production. Slavol is a preparation containing bacteria (nitrogen fixers and phosphomineralizers), plant growth stimulants that produce auxins (indole-3 acetic acid) in the fermentation process in the range of 0.01 to 0.1 mg/l. It affects cell division, stem and coleoptile growth, development of adventitious and lateral roots, flowering and pollination, and fruit quality.

Fitofert hemisuper plus organic-inorganic NPK fertilizer is used for all plant species, and exclusively for leafy vegetables grown on different soils in the field and substrates.

Second tested factor (B) includes the following variants: b_1) Santoro RZ, and b_2) Kiribati RZ.

In the stage of tenological maturity of lettuce we analyzed:

–weight of lettuce (g),

–vitamin C content in fresh leaf (mg100g^{-1}),

–dry matter content in the leaf (%).

The dry matter content in the leaf was determined by drying in an oven at 105°C. Based on the difference between the initial mass of the sample and after drying, the percentage of dry matter was calculated.

Vitamin C was determined by titration method. The significance of the differences of the environments was tested by the two-factorial variance analysis (ANOVA) method using SPSS 4.5 software.

Soil characteristics of the experimental field

Chemical analysis of the soil showed that the soil is an alkaline reaction, low in carbonate and belonging to the group of poorly lime soils, rich in humus, rich in nitrogen, very rich in readily available phosphorus and very rich in readily available potassium.

Table 1. Chemical properties of soil

pH H_2O	pH KCl	$CaCO_3$ %	Humus %	N %	P_2O_5 mg100g^{-1}	K_2O mg100g^{-1}
8,01	7,42	<1%	7,24	0,47	>40	65,41

RESULTS AND DISCUSSION

Weight of lettuce (g)

From the analysis of variance it can be concluded that the fertilization factor had a significant effect on the tested characteristic.

Table 2. Effect of fertilization and variety on the weight of lettuce (g)

Fertilization	Variety		Average for fertilization
	b_1	b_2	
a_1	169,49	135,09	152,29
a_2	268,90	349,48	309,19**
a_3	222,98	181,13	202,06
Average for variety	220,46	221,90	221,18

LSD	A	B	AxB
0,05	85,36	69,70	120,73
0,01	121,34	99,09	171,62

The highest weight of lettuce was recorded on variant a_2 (309.19 g) and compared with the control (152.29 g) and a_3 variant (202.06 g) the difference was statistically significant. Željkovic et al. (2013) confirmed the positive impact of

biostimulant application on the vegetative growth and chemical composition of marigold (*Tagetes patula* L.) belonging to the same family (fam. Asteraceae) as salad.

Similar results are reported by Đorđević et al. (2004) who emphasized that the use of Slavol microbial fertilizer in the production of pepper seedlings significantly influenced the increase in root length and above-ground part of the plant relative to the control variant. The group of authors concludes that the application of microbiological fertilizer stimulates the growth of the aboveground part of the plant by an average of 29% compared to the control variant (Bošković 2010; Govedarica et al., 1998; Đukić et al., 2007; Gecić et al., 2007). This effect of the microbiological fertilizer is explained by the ability of the nitrogen fixers to produce certain physiologically active substances such as auxin, gibberellin, cytokinin and vitamins. They stimulate the respiration energy of plant tissue, the activity of many enzymes, the process of photosynthesis, the absorption of water and minerals.

Dry matter content (%)

The average percentage of dry matter in a salad was in the range of 4 to 9%. In our studies, the dry matter content was from 5.10% to 6.46%.

Table 3. Dry matter content of lettuce leaf (%)

Fertilization	Variety		Average for fertilization
	b_1	b_2	
a_1	5,10	5,59	5,34
a_2	5,40	6,46	5,93**
a_3	5,69	6,01	5,85
Average for variety	5,39	6,02**	5,70

LSD	A	B	AxB
0,05	0,062	0,051	0,287
0,01	0,088	0,072	0,408

The highest percentage of dry matter was achieved in the second fertilizer variant (a_2-Slavol) in the Kiribati RZ variety - 6.46%, while the lowest percentage was achieved in the control variant (a_1 in the Santoro RZ variety-5.10%. The results of our research show that the dry matter content of salad is directly dependent on fertilization. Similar results are shown by Čabilovski et al. (2010). Also, Parađiković et al. (2009) conclude that% of dry weight of marigold (*Tagetes sp.*) root and leaves was significantly influenced by biostimulator treatment.

Vitamin C

Within the fertilization factor (Table 4), the highest content of vitamin C was found in the control variant (17.78 mg $100g^{-1}$) and the lowest in the variant a3 (10.01 mg 100g-1).

Tabela 4. Vitamin C content in lettuce leaf (mg $100g^{-1}$)

Fertilization	Variety		Average for fertilization
	b_1	b_2	
a_1	23,05	12,52	17,78**
a_2	14,91	7,50	11,02
a_3	12,02	8,01	10,01
Average for variety	16,66**	9,34	12,93

LSD	A	B	AxB
0,05	0,403	0,330	0,570
0,01	0,573	0,469	0,811

The results show that fertilization adversely affected the vitamin C content of the salad. Plakalovic (2018) has similar results. In his research, the author examined the impact of different fertilizer variants ($N_{120}P_{100}K_{120}$; $N_{120}P_{100}K_{120}$ + 30g Fitofert 20: 20: 20 / 100m² / day; $N_{120}P_{100}K_{120}$+ 40g Fitofert 4: 10: 40 / 100m² / day) on the qualitative components of young onions. The cited author concludes that increased amounts of fertilizers do not positively affect the vitamin C content. Similar results are shown by Premuzic et al. (2001) who found that N or bio stabilized compost fertilization does not change lettuce vitamin C content.

CONCLUSIONS

Based on the results of research on the effect of fertilization on the growth and quality of lettuce, it can be concluded:

–The application of microbial fertilizer had a stimulating effect on the growth of the above-ground part of the lettuce.

–The tested varieties of fertilizers with microbiological fertilizer achieved a statistically significantly higher mass of the aboveground part compared to the control variant.

–The dry matter content of the varieties did not different.

–Different fertilization methods did not positively affect the vitamin C content of lettuce. The highest content was recorded on the control fertilizer variant and the lowest content on the variant where organic-inorganic NPK fertilizer was applied.

REFERENCES

Adeoluwa, O., Adeogun, O.O. (2010). Evaluation of feather as organic fertilizers on Amaranthus *(Amaranthus caudatus).* In Proceedings of the 1st Technical Workshopon Organic Agriculture Conference, pp. 16-19. Ladoke Akintola University of Technology, Ogbomoso. Nigeria.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A.(2015): Biostimulants and crop responses: a review. Biological Agriculture and Horticulture 31, 1–17.

Bošković L. (2010). Influence of biofertilizers on soil microbial activity and plant growth *Camellia sp.* and *Cupressus macrocarpa Goldcrest*. Master's thesis. University of Montenegro. Faculty of Science. Podgorica.

Chaterjee, B., Ghanti, P., Thapa, U. and Tripathy, P. (2005). Effect of organic nutrition in sprouting broccoli *(Brassica oleracea L. var. italica Plenck)*. Vegetable Science 33 (1): pp 51-54.

Chatterjee, R., Bandhopadhyay, S. and Jana, J.C. (2014).Organic amendments influencing growth, head yield and nitrogen use efficiency in cabbage *(Brassica Oleracea Var.Capitata L.)* American International Journal of Research In Formal, Applied & Natural Sciences 5 (1):90-95

Čabilovski, R., Manojlovic, M., Bogdanovic, D. (2010). Monitoring of mineral nitrogen dynamics in soil and adoption by lettuce crop after application of organic fertilizers. Yearbook of scientific papers of the Faculty of Agriculture in Novi Sad. Year 34, no. 1. 46-52

Dauda, S.N., Ajayi, F.A. and Ndor, E. (2008). Growth and yield of water melon *(Citrullus lanatus)* as affected by poultry manure application Journal of Agriculture and Social Science, 4: 121 - 124

Đordjević, S., Jakovljevic, M., Santric, L. (2004). Effects of bacterialisation of pepper by the fertilizer "Slavol" in seedling production, Proceedings of the Scientific Papers, Vol.10, no. 1, 115-120

Đukić D.A., Jemcev V.T., Kuzmanova J. (2007). Soil biotechnology "Future", Novi Sad, p.529

Govedarica, M., Milosevic, N., Djukic, D.A., Mandic, L.G. (1998). Effect of *Azotobacter chroocroccum* and *Azospirillum lipoferum* strains on sugar beet yield and soil microbial activity, Actra Agriculturae Serbica, Vol.III, num. 5,pp 29-37.

Gecic, J., Mrkovacki, N., Cacic, N. (2007). Application of different types of sugar beet inoculation with *Azotobacter chroocroccum*, Annual of scientific papers, Volume 31, No. 1, 47-54

Makinde, E.A., Ayoola,O.T. and Akande, M.O. (2007). Effects of organic-mineral fertilizer application on the growth and yield of "egusi" melon *(Citrullus vulgaris L.)*. Australian Journal of Basic and applied sciences, 1(1): 15 – 19

Masarirambi, M. T., Hlawe, M. M., Oseni, O. T., Sibiya, T. E. (2010): Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (*Lactuca sativa* L.) 'Veneza Roxa'. Agriculture and Biology Journal of North America 1, 1319–1324.

Ndaeyo, N.U., Ukpong, E.S. and John, N.M. (2005). Performances of okra as affected by organic and inorganic fertilizers on an ultisol. Proceedings of the 39th Conference of the Agricultural Society of Nigeria October 9 – 13, p. 206 - 209.

Ogbonna P.E. (2008). Effectof combined application of organic and inorganic fertilizers on fruit yield of egg plant *(Solanum melongena)*. Pro. 42nd Annual conf. Agricultural Society of Nigeria (ASN) October 19-23 p. 236-250

Olowoake A.A., Adeoye G.O. (2010). Comparative eficacy of NPK fertilizer and composted organic residues on growth, nutrient apsorption and dry matter accumulation in maize. International Journal of Organic Agriculture Research and Development, vol. 2, pp. 43-53

Parađiković, N., Zeljković, S., Đurić. G., Vinković, T., Mustapić-Karlić, J., Kanižai, G., Iljkić, D. (2009). Growth and development of velvet (Tagetes erecta L.) under the influence of substrate volume and biostimulator treatment. In Proceedings of the 44th Croatian and 4th International Symposium of Agronomists, Lončarić, Z., Marić, S. (ed.), 786-790, Osijek, Croatia, Faculty of Agriculture in Osijek.

Plakalovic, J. (2018). Influence of agro-technical measures on the vulnerability and yield of young onions. Master thesis. University of East Sarajevo. Agricultural faculty.

Premuzic, Z., Garate, A., Bonilla, I. (2001): Yield and quality of greenhouse lettuce as affected by form of N fertiliser and light supply. In: Horst, W. J., Schenk, M. K., Bürkert, A., Claassen, N., Flessa, H., Frommer, W. B., Goldbach, H., Olfs, H. W.,Römheld, V., Sattelmacher, B., Schmidhalter, U., Schubert, S.,von Wirén, N., Wittenmayer, L. (eds.), Plant nutrition: food security and sustainability of agro-ecosystems through basic and applied research, 300–301. Kluwer Academic Publishers,Springer Netherlands.

Raševic, I. (2017). Effect of increasing doses of nitrogen on the chemical composition of carrots. Master thesis.Faculty of Agriculture, University of East Sarajevo.

Željković, S., Parađiković, N., Vinković, T., Tkalec, M., Maksimović, I., Haramija, J. (2013). Nutrient status, growth and proline concentration of French marigold *(Tagetes patula L.)* as affected by biostimulant treatment. Journal of Food, Agriculture & EnvironmentJFAE, WFL Publisher, Helsinki, Finland, 11(3&4), 2324-2327

Mori, Y., Tanaka, A., Nakagawa, T., Amen, Y., Kuwano, Y., Tanizaki, Y., Tomokiyo, S., Shimizu, K. (2020): Isolation and quantification of the plant growth regulator 1-triacontanol from Moso bamboo (Phyllostachys pubescens) shoot skin and its compost. Agriculture and Forestry, 66 (3): 81-93.

DOI: 10.17707/AgricultForest.66.3.08

Yasuhiro MORI[1], Akinobu TANAKA[2], Toshinori NAKAGAWA[2,3], Yhiya AMEN[2,4], Yasumitsu KUWANO[1], Yufu TANIZAKI[1], Shota TOMOKIYO[1], Kuniyoshi SHIMIZU[2]

ISOLATION AND QUANTIFICATION OF THE PLANT GROWTH REGULATOR 1-TRIACONTANOL FROM MOSO BAMBOO (*Phyllostachys pubescens*) SHOOT SKIN AND ITS COMPOST

SUMMARY

To investigate chemical uses of Moso bamboo (*Phyllostachys pubescens*) shoot skin, we identified the main component of non-polar solvent extracts. To this end, a white precipitate from *n*-hexane extracts was evaluated using silica-gel column chromatography. The fraction with the highest recovery showed a single spot in silica-gel thin-layer chromatography (TLC) analyses. In subsequent nuclear magnetic resonance (NMR) and electron impact-mass spectrometry (EI-MS) analyses, we identified the compound in the fraction as 1-triacontanol, which is a known regulator of plant growth. In addition, gas chromatography-mass spectrometry (GC-MS) experiments showed 1-triacontanol concentrations of 13.3 and 41.7 ppm in fresh and boiled skins, respectively. In boiled skins, 1-triacontanol concentrations reached a maximum of 71.3 ppm after 2 weeks of composting. Although concentrations gradually decreased thereafter, they remained at 19.7 ppm after compost maturation for 6 months. In a further experiment, seeds of Welsh onion were sown on absorbent cotton impregnated with authentic 1-triacontanol solutions, significant increase in hypocotyl length was observed. Due to the presence of 1-triacontanol, Moso bamboo shoot skin has potential as functional compost that promotes plant growth for agricultural uses.

Keywords: 1-triacontanol, compost, Moso bamboo shoot skin, non-polar solvent extract, *Phyllostachys pubescens.*

[1]Yasuhiro Mori, (corresponding author: drmorich@farc.pref.fukuoka.jp), Department of Biomass Utilization, Institute of Agricultural and Forest Resources, Fukuoka Agriculture and Forestry Research Center, 1438-2, Toyoda Yamamoto-machi, Kurume, Fukuoka 839-0827, JAPAN.
[2]Division of Sustainable Bioresources Science, Department of Agro-Environmental Sciences, Faculty of Agriculture, Graduate School of Kyushu University, Fukuoka, JAPAN.
[3]Department of Biological Resources Management, The University of Shiga Prefecture, Hikone, JAPAN.
[4]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, EGYPT.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:26/06/2020 *Accepted:23/07/2020*

INTRODUCTION

Bamboo shoots are a popular delicacy with a crunchy texture and pleasant taste, and are known as the King of Forest Vegetables (Chongtham et al., 2011). Annual consumption of bamboo shoots has been maintained at approximately 200,000 t for the past forty years in Japan (Forestry Agency, Ministry of Agriculture, Forestry and Fisheries, Japan, 2020). Moso bamboo (*Phyllostachys pubescens*) is the highest yield species of bamboo shoots in Japan (Torii and Isagi, 1997). Moso bamboo shoots are mainly available in fresh and canned forms. When harvested during winter (off-season), Moso bamboo shoots are marketed as the fresh form without peeling. Spring (peak season) harvests, however, are often processed by boiling and canning for long term storage. Boiled shoots are generally peeled prior to canning, leading to the accumulation of numerous shoot skins at canning factories during the peak season. Because they generate an unpleasant odor with decay, their disposal demands significant time and cost.

In our previous studies of Moso bamboo shoot skins, components from fresh skins were roughly divided into polar and non-polar compounds by extracting with *n*-hexane and then extracting the insoluble fractions with methanol and dichloromethane (Tanaka et al., 2011, 2013). We then examined the functions of polar solvent-extracts and isolated the compounds stigmasterol and dihydrobrassicasterol from dichloromethane-soluble fractions of methanol extracts. These compounds had antibacterial activity against *Staphylococcus aureus* (Tanaka et al., 2011, 2013).

Moreover, in an *in vitro* immunization system (Iwamoto et al., 2013), these methanol extracts suppressed immunoglobulin E production following stimulation by the cedar pollen antigen (Cry j 1) in human peripheral blood mononuclear cells (Tanaka, 2013). Antioxidant activities of methanol extracts were also described in oxygen radical absorption capacity assays and inhibition of melanin biosynthesis was demonstrated in cultured B-16 melanoma cells (Tanaka, 2013).

These studies collectively indicate that Moso bamboo shoot skins have functional ingredients with promise in cosmetics and other health-related products following extraction with polar solvents, such as methanol. Compounds that were extracted with the non-polar solvent *n*-hexane had limited antibacterial, antiallergic, antioxidant, and antimelanoma activities (Tanaka, 2013).

In this study, we further investigated functions of non-polar solvent extracts from Moso bamboo shoot skins by isolating major components of *n*-hexane extracts and identified the plant growth regulator 1-triacontanol (Ries et al., 1977b; Naeem et al., 2012). We also determined 1-triacontanol concentrations in composted boiled (hot water-extracted) shoot skins. Finally, we report the effects of 1-triacontanol treatments on seed germination and hypocotyl lengths of a plant species.

MATERIAL AND METHODS

Moso bamboo shoot skin and composting

Moso bamboo (*P. pubescens*) shoots were harvested in Miyako or Yame, Fukuoka prefecture, Japan, and non-boiled or boiled skins were provided by Life Design Co., Ltd. (Fukuoka, Japan) or Kazue Bussan Inc. (Fukuoka, Japan). Boiled skins (about 1 m^3) were composted in a pile on a composting platform at Fukuoka Agriculture and Forestry Research Center. The pile was turned manually every 2 weeks to maintain aerobic conditions and was watered when necessary. Samples were taken before boiling (fresh) and after 0, 1, and 2 weeks, and 2, 3, and 6 months after composting. Samples were roughly crushed into small pieces and were lyophilized after storage at −30°C.

Isolation and identification of the main components in n-hexane extracts from Moso bamboo shoot skin

Non-boiled (fresh) skins of Moso bamboo shoots were dried at room temperature and were then ground to powder. The powder (17.8 kg) was extracted with 72.5 l of *n*-hexane for 48 h. The *n*-hexane solution was then evaporated, yielding 40.1 g (dry weight) of extract. A 39 g sample of the extract was dissolved in 500 ml of *n*-hexane by sonicating and heating. The resulting solution was stored overnight at −20°C and was then decanted and the precipitated solute was purified to a yellowish crude powder.

The dissolving and decanting process was repeated until the solute was bleached and 5 g of white precipitate was finally obtained. Subsequently, 4 g of crude precipitate was applied to silica-gel column chromatography (column, φ 8.0 × 32.0 cm) with 800 g of Wakogel C-200 and was eluted with 2000 ml of *n*-hexane:ethyl acetate mixtures at ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 2.5:7.5, and 0:10, and then with methanol (2000 ml). For each elution stage, 500 ml fractions were collected, leading to a total of 36 fractions (fl–f36). Fractions were combined to form 12 fractions as follows: F1 (f1–f4, 8.1 mg), F2 (f5–f8, 7.2 mg), F3 (f9–f11, 473.7 mg), F4 (f12–f13, 641.9 mg), F5 (f14–f16, 325.0 mg), F6 (f17–f19, 290.1 mg), F7 (f20–f21, 32.2 mg), F8 (f22–f24, 63.8 mg), F9 (f25–f28, 50.2 mg), F10 (f29–f32, 22.1 mg), F11 (f33–f34, 2.4 mg), and F12 (f35–f36, 106.1 mg). These fractions were analyzed using silica-gel thin-layer chromatography (TLC) on silica-gel 60 F254 plates (Merck Co., Darmstadt, Germany) with *n*-hexane:ethyl acetate:acetic acid at 9:1:0.1 (F1–F3), 7:3:0.1 (F4–F8), or 6:4:0.1 (F9–F12). Spots were made visible by spraying with 10% sulfuric acid/methanol and then heating on a hot plate to 140°C. Among fractions, F4 was recovered with the highest quantity and contained a pure compound that formed a single spot in TLC analyses.

The chemical structure of the compound was analyzed using nuclear magnetic resonance (NMR) and electron impact-mass spectrometry (EI-MS). ^{1}H and ^{13}C-NMR spectra were generated on a Bruker DRX 400 NMR spectrometer (Bruker Daltonics Inc., MA, USA) using tetramethylsilane (TMS) as an internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm with

reference to TMS resonance. EI-MS was performed using a JEOL JMS 700 spectrometer (JEOL, Japan). Organic solvents and Wakogel C-200 were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Chloroform extraction from Moso bamboo shoot skin

Lyophilized samples were ground into 1-mm particles using a Wiley mill and were extracted with chloroform, because authentic 1-triacontanol (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was more soluble in chloroform than in *n*-hexane in our preliminary tests. Ground samples (10 g) were extracted three times with 100 ml of chloroform at room temperature with shaking at 160 rpm for 24 h. After dehydration with anhydrous sodium sulfate, solutions were filtered through filter paper (No. 5A, 150 mm; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and were concentrated using a rotary evaporator. Dried extracts were redissolved in chloroform to a concentration of 5 mg/ml.

Quantitative gas chromatography-mass spectrometry analysis of 1-triacontanol

Forty-microliter aliquots of *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA; Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) were added to each chloroform extract (160 µl), and derivatization was achieved by heating the mixtures at 60°C for 10 min. Gas chromatography-mass spectrometry (GC-MS) analyses were performed using an Agilent 7890A gas chromatograph (Agilent Technologies, Inc., CA, USA) equipped with an Agilent 7693A autosampler (Agilent Technologies, Inc., CA, USA) and a Agilent 5975C inert XL MSD mass selective detector (Agilent Technologies, Inc., CA, USA). Into a DB-5MS capillary column (30 m long, 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Inc., CA, USA) in the splitless mode, 1 µl aliquots of derivatized sample were injected. The oven temperature was operated at 40°C for 3 min, and the temperature was then increased to 300°C at 15°C/min and was held at this temperature for 10 min. Helium was used as a carrier gas. Injector and detector temperatures were both 250°C. 1-Triacontanol was identified as a trimethylsilyl derivative by comparing retention times and mass spectra with those from the NIST 08 library and an authentic compound in total ion monitoring (SCAN) mode. Ions at *m/z* 471 and 495 were chosen as targets for identification and quantification of 1-triacontanol in selected ion monitoring (SIM) mode, because they were the most abundant and lacked cross-interferences in mass spectra. In SIM mode, 1-triacontanol concentrations were quantified using an external standard method with a 5-point calibration curve and peak areas of authentic standards ranging from 25 to 400 µg/ml.

Plant growth test

Solutions of authentic 1-triacontanol were adjusted to 1000 ppb in distilled water with 0.1% Tween20, and were then autoclaved at 121°C for 20 min. They were serially diluted to 1, 10, and 100 ppb in distilled water. Thirty Welsh onion

(*Allium fistulosum*) seeds were sown on absorbent cotton impregnated with 40 ml of each solution using a Germination Index Kit (JPec Co., Ltd., Tokyo, Japan). After incubation for 6 days in the dark at 25°C, germination rates and hypocotyl lengths were measured. The test was independently repeated three times.

RESULTS AND DISCUSSION

Isolation and identification of 1-triacontanol from n-hexane soluble fractions of Moso bamboo shoot skins

Silica-gel column chromatography of white precipitates from *n*-hexane extracts of fresh Moso bamboo shoot skins were collected in 12 fractions. A single spot from the fraction (F4) on TLC plates showed the highest recovery (641.9 mg) of all fractions. This spot was also detected in TLC analyses of the other four fractions (F5 to F8), with close polarities to that of F4 (data not shown). Thus, the main component of the white precipitate was isolated from F4 and was considered a pure compound.

The pure compound 1 was isolated as a white powder from F4 (Fig. 1). ^{13}C spectrum signals were discriminated into CH_3 resonance at δ_C 14.3 and a signal due to a primary alcoholic group at δ_C 63.3 (Fig. 2). Other signals were assigned to aliphatic CH_2 groups, which resonated at δ_C 22.9–33.0. These data suggested that compound 1 is an aliphatic straight chain primary alcohol. ^1H-NMR spectrum revealed a signal at δ_H 3.64 (t, J = 6.8 Hz), indicating a CH_2OH group, and a signal at δ_H 0.88 (t, J = 8.0 Hz, 3H) for a terminal CH_3 group (Fig. 3). An upfield broad signal resonated at δ_H 1.25 (54H, m) and was assigned to H3–H29.

The number of protons at δ_H 1.25 was assumed based on the integrated intensity of the terminal methyl at δ_H 0.88 and this was confirmed using EI-MS (Fig. 4). In the splitting pattern, a triplet peak at δ_H 0.88 was integrated for 3H and indicated the presence of CH_3CH_2 as a partial structure of the compound. Another triplet peak with 2H at δ_H 3.64 was considered to be from an α-hydrogen adjacent to a hydroxyl group, indicating the presence of CH_2CH_2OH. A broad peak with 54H at δ_H 1.25 and a multiplet peak with 2H at δ_H 1.57 indicated the presence of $[CH_2]_{27}$ and CH_2, respectively. Correlated spectroscopy spectra showed correlations between protons at δ_H 0.88 and δ_H 1.25, at δ_H 1.25 and δ_H 1.57, and at δ_H 1.57 and δ_H 3.64 (Fig. 5).

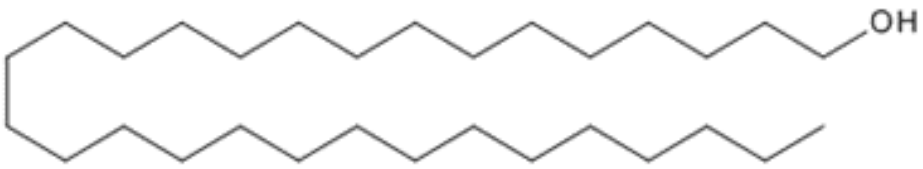


Fig. 1. Chemical structure of 1-triacontanol ($C_{30}H_{62}O$).

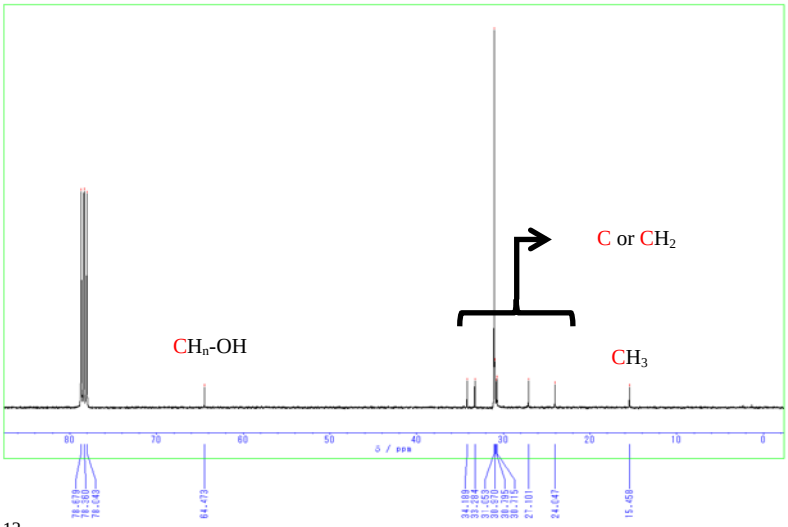


Fig. 2. ^{13}C nuclear magnetic resonance (NMR) spectra of the fraction F4 from *n*-hexane extracts of fresh Moso bamboo shoot skins; herein, chloroform appeared in a triplet peak with a chemical shift at 78.36 ppm. Because this value is usually 77.2 ppm, presented x values were adjusted by −1.16 ppm.

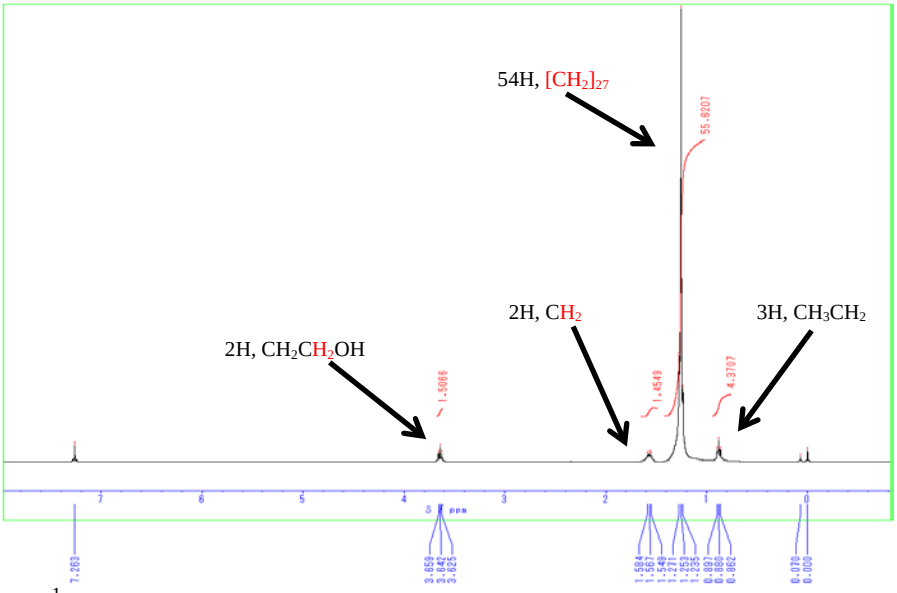


Fig. 3. ^{1}H nuclear magnetic resonance (NMR) spectra of fraction F4 of *n*-hexane extracts from fresh Moso bamboo shoot skins.

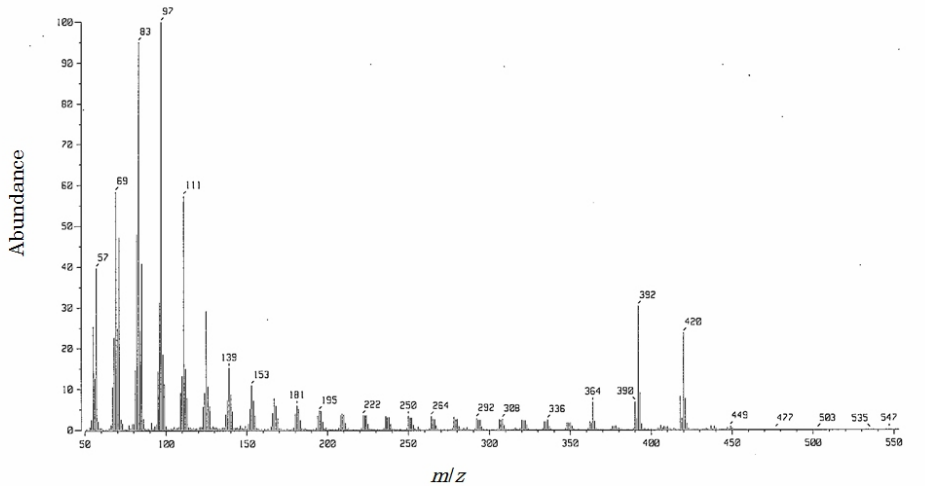


Fig. 4. Electron impact-mass spectrometry (EI-MS) of fraction F4 of *n*-hexane extracts from fresh Moso bamboo shoot skins.

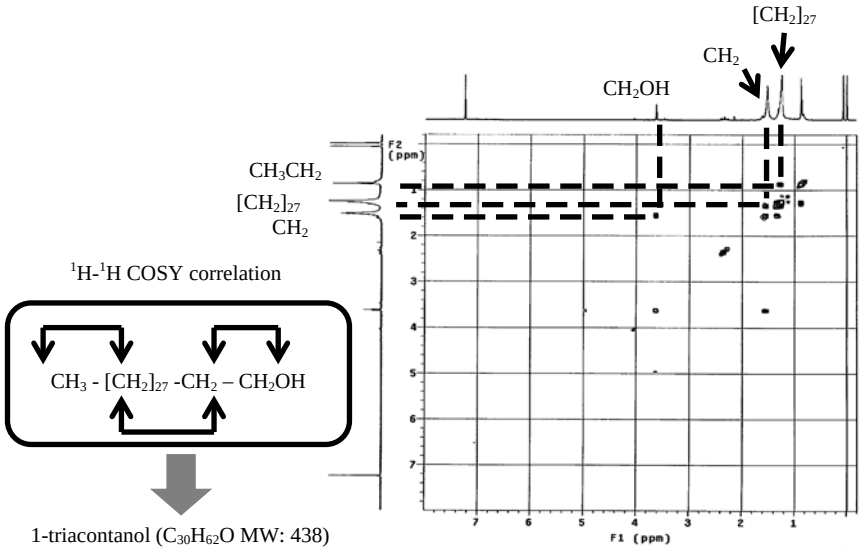


Fig. 5. ^{1}H-^{1}H correlated spectroscopy spectra of fraction F4 of *n*-hexane extracts from fresh Moso bamboo shoot skins.

Connection of the partial structures based on these correlations strongly suggested that the compound in F4 was 1-triacontanol. EI-MS spectra showed a base peak at *m*/*z* 420 $[M\text{-}H_2O]^+$, thus confirming the molecular formula $C_{30}H_{62}O$ and the molecular weight of 438 g/mol (Fig. 4). This base peak also supported the presence of a hydroxyl functional group in the compound. Data from NMR and EI-MS analyses of compound 1 were matched with those reported previously (Du et al., 2009; Upadhyay et al., 2006; Randrianasolo et al., 2015). Chibnall et al.

(1931) was the first to discover 1-triacontanol, and described the compound as a constituent of apple peel wax. Subsequently, this compound was isolated from epicuticular waxes of alfalfa leaves (Chibnall et al., 1933), rice leaves (Uchiyama and Ogasawara, 1981), soybean (*Glycine max*) leaves (Hagedorn et al., 2017), and from sub-epidermal cells of jade plant (*Crassula argentea*) leaves (Kolker, 1978), potato (*Solanum tuberosum*) tubers (Kolker, 1978), and beeswax (Jackson and Eller, 2006). We are the first to report 1-triacontanol in Moso bamboo shoot skins.

Quantification of 1-triacontanol from Moso bamboo shoot skins during composting

Concentrations of 1-triacontanol in dry matter non-boiled (fresh) and boiled Moso bamboo shoot skins during composting are shown in Fig. 6. Before composting, boiled shoot skins contained 1-triacontanol at 41.7 ppm, more than threefold higher than in non-boiled skins (13.3 ppm). With progress of composting, 1-triacontanol concentrations significantly increased and peaked at 71.3 ppm after 2 weeks. Thereafter, 1-triacontanol concentrations gradually decreased to 19.7 ppm after 6 months of composting.

According to quantitative determinations by Kolker (1978), 1-triacontanol concentrations in rice, maize (*Zea mays*), and alfalfa leaves were 481, 234, and 173 ppm, respectively, much higher than in fresh Moso bamboo shoot skins (13.3 ppm) and boiled skins (41.7 ppm) (Fig. 6). According to assessments from the Organization for Economic Co-operation and Development (OECD), alcohols with carbon chain lengths up to C16 are readily biodegradable, with 100% degradation in less than 10 days (OECD, 2006). In addition, alcohols with chain lengths of C16, C18, and over C18 were degraded by 62%, 76%, and 37%, respectively (OECD, 2006). In the present study, 1-triacontanol concentrations in boiled skins increased to 71.3 ppm after 2 weeks of composting (Fig. 6). Hence, we suggest that the long chain length (C30) of 1-triacontanol may be more resistant to degradation than alcohols with shorter chain lengths. Therefore, various low-molecular weight compounds, including those from hot-water extracts, are thought to have been preferentially degraded. We observed gradual decreases in 1-triacontanol concentrations with further composting, with a remaining concentration of 19.7 ppm at 6 months (Fig. 6). In assessments of germination rates of komatsuna (*Brassica rapa* var. *perviridis*) using Germination Index Kits, boiled skins needed 4 months for compost maturation (Tanizaki, unpublished data).

Thus, mature composts of boiled skins could be useful as functional composts, because they do not negatively affect plant growth by limiting nitrogen and contain the plant growth regulator 1-triacontanol.

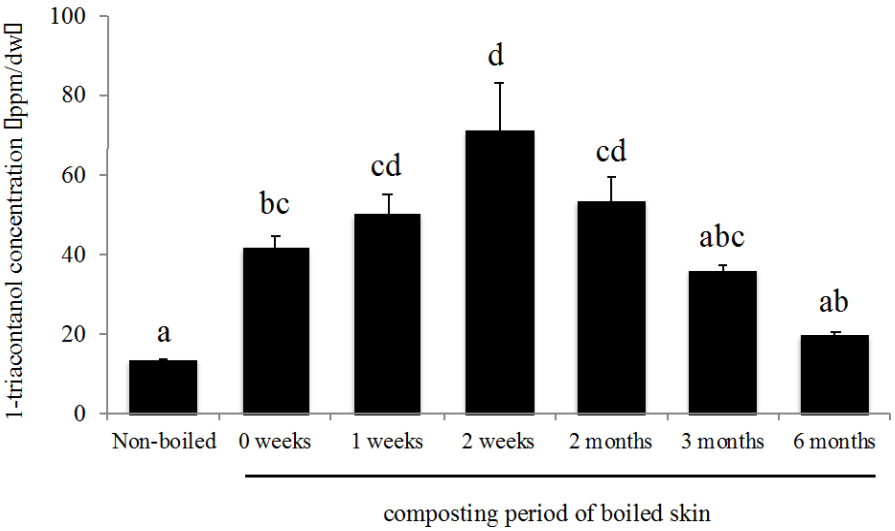


Fig. 6. Concentrations of 1-triacontanol in Moso bamboo shoot skins; bars indicate mean concentrations of 1-triacontanol per dry weight of skins (mean ± SE, *n*=3). Different lower-case letters indicate significant differences in concentrations ($p < 0.05$), as indicated by Tukey's tests.

Effects of 1-triacontanol treatments on plant growth

In this study, we monitored germination rate and hypocotyl length of Welsh onion after treatments with authentic 1-triacontanol solutions (Table 1). Germination rates of Welsh onion were over 90% in all of these treatment conditions except in the presence of 10 ppb 1-triacontanol. Yet hypocotyl lengths of Welsh onions were significantly greater (1.25 times) in 1000 ppb 1-triacontanol solution than in controls. No significant effects of 1-triacontanol treatments on Welsh onions were observed at lower concentrations.

Table 1. Germination rate and hypocotyl length of Welsh onion after treatment with authentic 1-triacontanol solutions at different concentrations

1-triacontanol concentration	Germination rate (%)				Hypocotyl length (mm)			
	mean		SE		mean		SE	
0ppb	96.7	±	0.0	ab	18.3	±	1.0	a
1ppb	98.9	±	1.1	a	20.1	±	0.9	ab
10ppb	88.9	±	2.9	b	17.8	±	1.2	a
100ppb	95.6	±	2.2	ab	20.3	±	0.9	ab
1000ppb	98.9	±	1.1	a	22.9	±	0.8	b

Different lower-case letters indicate significant differences in germination rates or hypocotyl lengths ($p < 0.05$) from Tukey's tests.

1-Triacontanol was originally isolated from chloroform extracts of alfalfa (*Medicago sativa*) as a plant growth regulator that promoted water uptake and dry weight of rice (*Oryza sativa*, Ries et al., 1977b). According to a review article (Naeem et al., 2012), 1-triacontanol enhances growth, yield, photosynthesis, and chlorophyll contents of various plant species. When applied to shoots of rice plants, 1-triacontanol rapidly elicited biosynthesis of 9-β-L(+)-adenosine in the roots (Ries, 1991; Naeem et al., 2012) as a probable source, adenosine monophosphate from adenosine diphosphate and adenosine triphosphate (Olsson and Pearson, 1990; Naeem et al., 2012). Subsequently, apoplastic ion concentrations increased in plant tissues (Ries et al., 1993; Naeem et al., 2012). In published suppression subtractive hybridization and Northern blotting analyses, 1-triacontanol upregulated photosynthetic and photorespiratory genes and downregulated abscisic acid and stress- and wound-related genes (Chen et al., 2002). These events are thought to trigger various metabolic activities, including photosynthesis, nutrient uptake, and enzyme activity, likely leading to accelerated plant growth.

In this study, 1-triacontanol significantly promoted hypocotyl lengths of Welsh onion, but did not affect germination at the tested concentrations (Table 1). When micro propagated balm (*Melissa officinalis*) was planted in medium supplemented with 1-triacontanol at 2–10 ppb, shoot lengths increased significantly (Tantos et al., 1999). Significantly enhanced shoot growth was also observed in herbal plants, including coriander (*Coriandrum sativum*, Idrees et al., 2010), sweet basil (*Ocimum basilicum*, Hashmi et al., 2011), and *Artemisia annua* (Aftab et al., 2010) following treatments with foliar spray containing 1-triacontanol at 439, 4.39, 43.9, and 1.5 ppm, respectively. In another study, seed germination of maize, paddy, and sunflower was significantly promoted by soaking in solution containing 1–10 ppm 1-triacontanol (Niranjana et al., 1999). In contrast, however, germination rates of seeds from 15 species, including soybean, lettuce (*Lactuca sativa*), and purslane (*Portulaca oleracea*), were not significantly enhanced when sown on absorbent paper in petri dishes containing 1-triacontanol at 4.39 ppm (Hoagland, 1980).

Thus, the plant growth promotion effect of 1-triacontanol likely depends on concentration, treatment mode, and species. In this study, we demonstrated that boiled skins of Moso bamboo shoot hold sufficient concentrations of 1-triacontanol to promote hypocotyl lengths of Welsh onion even after compost maturation. Further studies are required to determine volumes of Moso bamboo skins that are required to improve soils and promote growth or improve yields of agricultural plants such as Welsh onion.

More evidence is required to confirm that 1-triacontanol was responsible for increased growth of the present plants. Increased yields of vegetable or crop plants following application of boiled Moso bamboo skins will set a precedent, as shown with alfalfa plants (Ries et al., 1977a). Discarded biomass from bamboo shoot production in a village could be converted to local compost contributing to agricultural production within the same village.

CONCLUSIONS

We isolated the main component of *n*-hexane extracts from fresh Moso bamboo shoot skins using silica-gel column chromatography and identified the plant growth regulator 1-triacontanol in NMR and EI-MS analyses. In our GC-MS analyses, 1-triacontanol concentrations remained 19.7 ppm even after boiled skins were composted for 6 months. Our laboratory experiments show that 1-triacontanol significantly promotes hypocotyl length of germinated Welsh onion. Thus, Moso bamboo shoot skins may provide functional compost that contains a plant growth regulator.

ACKNOWLEDGEMENTS

We thank Shojiro Oda (Life Design Co., Ltd., Fukuoka, Japan) and Takahiro Kazue (Kazue Bussan Inc., Fukuoka, Japan) for providing Moso bamboo shoot skins. The assistance of Akira Shima, Akihiro Tsutsumi, Michiyo Yamamoto, Mayumi Ikeda, Kuniko Okubo, and Yuri Uchida (Institute of Agricultural and Forest Resources, Fukuoka Agriculture and Forestry Research Center) in extracting from Moso bamboo shoot skins is greatly appreciated. We would like to thank Enago (www.enago.jp) for the English language review. Finally, the anonymous reviewers are gratefully acknowledged for valuable comments and suggestions.

REFERENCES

Aftab T, Khan MMA, Idrees M, Naeem M, Singh M, and Ram M. 2010. Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. Journal of Plant Interactions, 5:273–281.

Chen X, Yuan H, Chen R, Zhu L, Du B, Weng Q, and Heet G. 2002. Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): Possible role of triacontanol as a plant growth stimulator. Plant and Cell Physiology, 43:869–876.

Chibnall AC, Piper SH, Pollard A, Smith JAB, and Williams EF. 1931. The wax constituents of the apple cuticle. Biochem Journal, 25:2095–2110.

Chibnall AC, Williams EF, Latner AL, and Piper SH. 1933. The isolation of *n*-triacontanol from lucerne wax. Biochem Journal, 27:1885–1888.

Chongtham N, Bisht MS, and Haorongbam S. 2011. Nutritional properties of bamboo shoots: Potential and prospects for utilization as a health food. Comprehensive Reviews in Food Science and Food Safety, 10:153–168.

Du S, Wang M, Zhu W, and Qin Z. 2009. A new fungicidal lactone from *Xylocarpus granatum* (Meliaceae). Natural Product Research, 23:1316–1321.

Forestry Agency, Ministry of Agriculture, Forestry and Fisheries, Japan 2020. A summary for demand and supply of main non-wood forest products in Japan fiscal year 2018. https://www.e-stat.go.jp/dbview?sid=0001784441. (accessed June 05, 2020) (In Japanese).

Hagedorn O, Fleute-Schlachter I, Mainx HG, Zeisler-Diehl V, and Koch K. 2017. Surfactant-induced enhancement of droplet adhesion in superhydrophobic soybean (*Glycine max* L.) leaves. Beilstein Journal of Nanotechnology, 8:2345–2356.

Hashmi N, Khan MMA, Naeem M, Idrees M, Aftab T, and Moinuddin 2011. Ameliorative effect of triacontanol on the growth, photosynthetic pigments, enzyme activities and active constituents of essential oil of *Ocimum basilicum* L. Medicinal and Aromatic Plant Science Biotechnology, 5:20–24.

Hoagland RE. 1980 Effects of triacontanol on seed germination and early growth. International Journal of Plant Sciences, 141:53–55.

Idrees M, Khan MMA, Aftab T, and Naeem M. 2010. Synergistic effects of gibberellic acid and triacontanol on growth, physiology, enzyme activities and essential oil content of *Coriandrum sativum* L. The Asian and Australasian Journal of Plant Science and Biotechnology, 4:24–29.

Iwamoto A, Mitsuda K, Inoue A, Kato T, Inoue Y, and Kawahara H. 2013. Purification and identification of an IgE suppressor from strawberry in an *in vitro* immunization system. Cytotechnology, 64:309–314.

Jackson MA, Eller FJ. 2006. Isolation of long-chain aliphatic alcohols from beeswax using lipase-catalyzed methanolysis in supercritical carbon dioxide. The Journal of Supercritical Fluids, 37:173–177.

Kolker LS. 1978. Analytical procedures for 1-triacontanol and its presence in plants and the environment. Dissertation, Michigan State University

Naeem M, Khan MMA, and Moinuddin 2012. Triacontanol: a potent plant growth regulator in agriculture. Journal of Plant Interactions,7:129–142.

Niranjana SR, Pandit A, Prakash HS, and Shetty HS. 1999. Effect of triacontanol on seed quality of maize, paddy and sunflower. Seed Science and Technology, 27: 1007–1013.

Olsson RA, Pearson JD. 1990. Cardiovascular purinoceptors. Physiological Reviews, 70:761–845.

Organization for Economic Co-operation and Development (OECD) 2006. Long chain alcohols (C6-22 primary aliphatic alcohols). SIDS (Screening Information Data Set) initial assessment report for SIAM (SIDS Initial Assessment Meeting) 22. Paris, France. 18–21 April.

Randrianasolo R, Krebs HC, Rakotoarimanga JV, Raharisolololao A, and Rakotondramanga M. 2015. Secondary metabolites of *Phellolophium madagascariensis*. Journal of Pharmacognosy and Phytochemistry, 4:55–56.

Ries SK, Bittenbender H, Hangarter R, Kolker L, Morris G, and Wert V. 1977a. Improved growth and yield of crops from organic supplements. In: Lockeretz W (ed) Agriculture and Energy. Academic Press, New York, pp 377–384.

Ries SK, Wert V, Sweeley CC, and Leavitt RA. 1977b. Triacontanol: A new naturally occurring plant growth regulator. Science, 195:1339–1341.

Ries SK. 1991. Triacontanol and its second messenger 9-β-L(+)- adenosine as plant growth substances. Plant Physiology, 95:986–989.

Ries S, SavithiryS, Wert V, and Widders I. 1993. Rapid induction of ion pulses in tomato, cucumber, and maize plants following a foliar application of L(+)-adenosine. Plant Physiology, 101:49–55.

Tanaka A, Kim HJ, Oda S, Shimizu K, and Kondo R. 2011. Antibacterial activity of moso bamboo shoot skin (*Phyllostachys pubescens*) against *Staphylococcus aureus.* Journal of Wood Science, 57:544–542.

Tanaka A. 2013. A search of bioactive substances from Moso bamboo. Master thesis, Kyushu University (in Japanese).

Tanaka A, Shimizu K, and Kondo R. 2013. Antibacterial compounds from shoot skins of moso bamboo (*Phyllostachys pubescens*). Journal of Wood Science, 59:155–159.

Tantos Á, Mészáros A, Kissimon J, Horváth G, and Farkas T. 1999. The effect of triacontanol on micropropagation of balm, *Melissa officinalis* L. Plant Cell Reports, 19:88–91.

Torii A, Isagi Y. 1997. Range expansion of bamboo species in southern areas of Kyoto prefecture, Japan. Japanese Journal of Ecology, 47:31–41. (in Japanese with English abstract)

Uchiyama T, Ogasawara N. 1981. Constituents of plant leaf waxes contained in rice callus tissues. Agricultural and Biological Chemistry, 45:1261–1263.

Upadhyay RK, Rohatgi L, Chaubey,MK, and Jain SC. 2006. Ovipositional responses of the pulse beetle, *Bruchus chinensis* (Coleoptera: Bruchidae) to extracts and compounds of *Capparis decidua*. Journal of Agricultural and Food Chemistry, 54:9747–9751.

Vajkić, M., Vrnješ, B., Stojiljković, E., Đapan, M. (2020): Occupational injury analysis in the forestry of the Republic of Srpska-Bosnia and Herzegovina. Agriculture and Forestry, 66 (3): 95-104

DOI: 10.17707/AgricultForest.66.3.09

Mile VAJKIĆ[1], Biljana VRANJEŠ[2], Evica STOJILJKOVIĆ[3], Marko ĐAPAN[4]

OCCUPATIONAL INJURY ANALYSIS IN THE FORESTRY OF THE REPUBLIC OF SRPSKA-BOSNIA AND HERZEGOVINA

SUMMARY

Forestry in the Republic of Srpska (BIH) is an important branch in industry, with the majority of activities in the field of forestry being conducted by the Public Forest Enterprise "Forests of the Republic of Srpska". From the aspect of occupational safety and health, forestry is a high-risk economic activity in which a large number of occupational injuries occur, often with fatalities. The basic task of occupational safety and health is to provide the best working conditions for all employees by designing occupational safety measures. To fulfil this task, the occupational injury analysis provides the opportunity to properly select preventive measures of protection and indicates the importance and magnitude of the problems related to occupational safety in the organization. Also, the analysis of certain etiological specificities of injury enables more accurate actions within the organization, with the aim of defining instructions for improving the risk management process in the organization. In this paper, occupational injury analysis in forestry was carried out in for the period 2011-2019., and obtained research results shows that 76% of injuries occur during woodcutting, and that about 60% of those injuries are related to the occupation of a cutter. Also, research see that the cause of injuries in 49% is the worker's negligence.

Keywords: forestry, occupational injury, risk management, occupational safety and health.

INTRODUCTION

Forestry, due to a large number of potential hazards and harms to the health and safety of workers, as well as the probability of accidents, is a challenge for occupational safety and health engineers to prevent occupational injuries and occupational deceases. Forest work is considered to be one of the most dangerous

[1]Mile Vajkić, (corresponding author: m.wajkic@gmail.com), High School of mechanical engineering, Prijedor, Republika Srpska, BOSNIA AND HERZEGOVINA.
[2]Biljana Vranješ, University of Banja Luka, Faculty of Mechanical engineering, Banja Luka, Republika Srpska, BOSNIA AND HERZEGOVINA.
[3]Evica Stoiljković, University of Niš, Faculty of Occupational Safety, Niš, SERBIA.
[4]Marko Đapan, University of Kragujevac, Faculty of Engineering, Kragujevac, SERBIA.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:04/05/2020 *Accepted:05/08/2020*

occupations in the world (Lilley et al., 2002; Klun and Medved, 2007). Forest workers can still be found not wearing the compulsory safety equipment and ignoring safety rules and thus, accident rates are fairly high. Neither do most forest workers use personal protective equipment, although it is recommended in order to avoid serious and fatal accidents (Yoshimura and Acar, 2004). Application of safety equipment, such as safety trousers, decreases the number of accidents with chain saw, but on the other hand it in-creases the number of injuries of other unprotected body parts (Sullman et al. 1999). Europe-wide, the number of fatal occupational accidents in the agricultural, hunting and forestry sectors is higher than in any other (Kogler et al., 2015).An occupational injury is a change occurred in the work environment affecting one's integrity (physical, psychological and social), it occurred as a consequence of the sudden and immediate inconsistency between the behaviour of the person in the performance of work activity and the elements of the work environment (Krstić I., Anđelković B., 2013). Felling, cross-cutting, extraction and skidding operations are the most common operations that result in fatal forestry accidents. Each of the harvesting methods also has its specific features that depend on natural and production conditions, the technology used, and the share of manual operations in the overall process (Gerasimov and Sokolov, 2014). Exposure to the harms, which are largely present in the forestry, causes the onset of occupational diseases. Occupational diseases are those diseases which are caused by harms at the workplace that are caused by the longer direct influence of the work process and workplace conditions (Krstić I., Anđelković B., 2013). The large number of injuries which often have a fatal outcome, as well as the occurrence of occupational diseases due to exposure to numerous harms, classify forestry as a high-risk industry. In the world, the number of occupational injuries in forestry exceeds 170 000 per year, while the number of fatal injuries ranges from 80 to 100 (Garland, J. J., 2018). Taking into account the facts, experiences and previous research in the field of occupational safety in forestry and collected data on accidents in The Public Forestry Enterprise "Forests of the Republic of Srpska", we decided to conduct this study to indicate the most important factors of accidents in forest operations. The aim of this analysis is to present the most common causes and sources of injuries at work in forestry in the Republic of Srpska (BIH) and to indicate the jobs where injuries most often occur. The results of this analysis contribute to a better organization of safety at work in forestry and enable more precise action in order to reduce risks in the workplace.

MATERIAL AND METHODS

Occupational injury analysis in forestry was carried out in The Public Forestry Enterprise "Forests of Republic of Srpska" for the period 2011-2019. The analysis was conducted on the basis of data obtained by the occupational safety service in The Public Forestry Company "Forests of Republika Srpska". Any injury (minor, severe, or fatal) in forest operations is recorded using special documents (report of injury). Report of injury contains information on the age

and qualification of the person involved in the accident, time and place of the accident, activities and phase of work at the time of the accident, form and distribution of injuries, severity of the accident, source and cause of the injury.

In the period 2011-2019, this company managed 77% of forest resources in Republika Srpska (BIH) and employs an average of 4,500 workers. The main harvesting technology was motor-manual processing with a chainsaw. In addition to felling and pruning wood, actions were carried out in order to protect forests, maintain roads and watercourses and afforestation. Tractors, loading machinery and trucks were used for transport. An injury risk assessment was performed for all workplaces and all forest workers had standard protective equipment. In this paper for the period 2011-2019. the following analyzes were performed:

- injuries by type of employment,
- injuries by gender of the injured worker,
- injuries by educational degree of the injured,
- injuries by the place of occurrence of the injury,
- injuries by the season when they occurred,
- injuries by the day of the week in which the injury occurred,
- injuries according to the job classification,
- sources and causes of occupational injuries.

RESULTS AND DISCUSSION

Fatal accident statistics are generally the most accurate of all accident statistics in that they are not reported by the injured person and must be published in official records (Thelin, 2002; Lindroos and Burstrom, 2010). According to occupational accident reports gathered from a number of selected International Labour Office member states, the average estimated fatal occupational accident frequency rate in forestry, logging and related services was 14/100,000 workers. (Enez et al.,2014). The EU collects data on occupational injuries from the Member States. It is considered that the number of occupational injuries with fatal outcome is reliable, although there are also different ways of statistical observation.

In the legislation of the Republic of Srpska (BIH), categorization of injuries by severity is: light, serious and fatal injuries. In the analyzed period (2011-2019), the RS forestry recorded 1265 minor injuries, 96 serious injuries and 8 fatal injuries. The smallest number of injuries was 133 in 2011, and the highest of 171 injuries at work was in 2017. For the period 2011-2019, the annual average number of injuries at work in RS is 1236, therefore occupational injuries in forestry with an average annual number of 152 make up 12% of total injuries in the Republic of Srpska. Figure 1. shows the number of injuries, classified by severity, in the Republic of Srpska forestry.

From Figure 1 we can conclude that in the Republic of Srpska forestry, there is a trend of the increase in the total number of injuries at work, while in the case of fatal injuries there is a trend of slight downward, Figure 1.

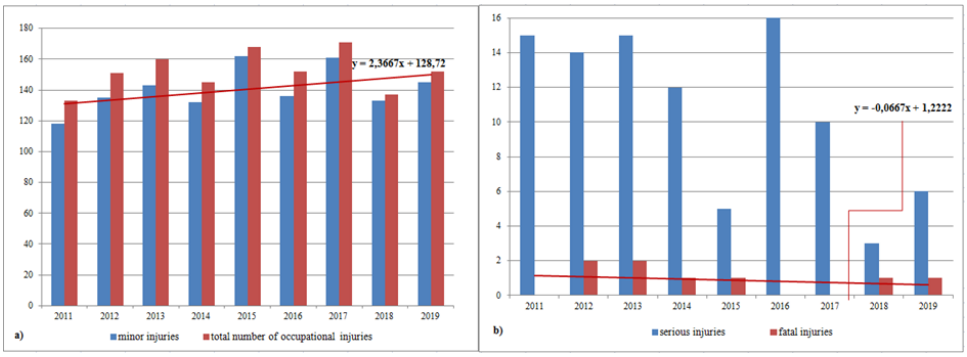


Figure 1. Overview of occupational injuries in the Republic of Srpska forestry for the period 2011-2019 (left-minor injuries and the total number of occupational injuries; Right -serious injuries and fatal injuries)

.

Occupational injury analysis involves analysis of the etiological components of injuries, such as gender, type of employment, educational degree, day of the week, place of occurrence, season, source and cause of the injury occurrence, etc. (Spasić, D., 2001.). An occupational injury analysis according to these etiological factors in the Republic of Srpska forestry is shown in Figure 2.

According to the data obtained on injuries in the RS forestry for the period 2011-2019, shown in the previous figure we conclude that: 97 % of all injuries occur during regular working hours, that the male labour force is the most injured (96%); that the most injured are workers with secondary education (47%) and skilled workers (38 %); that the highest number of injuries occurs during cutting, felling, skidding and loading of wood (80%); that injuries mostly happen during summer (33%) and on Mondays (24%). On the basis of the conducted research the result of occupational injuries by gender is expected because the male labour force is predominantly employed in this industry (Employees in the Public Forestry Company "Forests of Republika Srpska" by sex 90% men and 10% women). Also, the majority of employees in this industry are skilled workers and workers with secondary education, therefore the highest percentage of injured is among this population. In comparison to the seasons, injuries most often occur in the summer, due to the favourable weather conditions for outdoor work, although the prevalence of injuries in other seasons is large and quite equable.

According to the results of the conducted research, the operation of the occupational safety system and the design of safety measures should be directed to work operations performed during cutting, felling, skidding and loading of wood during regular working hours.

Also, the data shows that most injuries occur on Mondays, almost ¼ of all injuries, i.e. 24%. This result can be explained by the inadequate use of free time during the weekend and the need to "break-in" in the work rhythm at the beginning of the workweek, which is strongly related to complex physical work, and this result is also present in the research of other authors (Šporčić, M., Sabo, A. 2002.; Musić, J. et al .2019.; Landekić, M. 2010). Organizational measures of

worker preparation in the form of brief meetings to remind workers of the importance of following work procedures and procedures with an emphasis on the importance of following safety rules and the proper use of personal protective equipment and means, with a slower work pace, should be used as a measure to reduce the number of injuries at the beginning of the workweek.

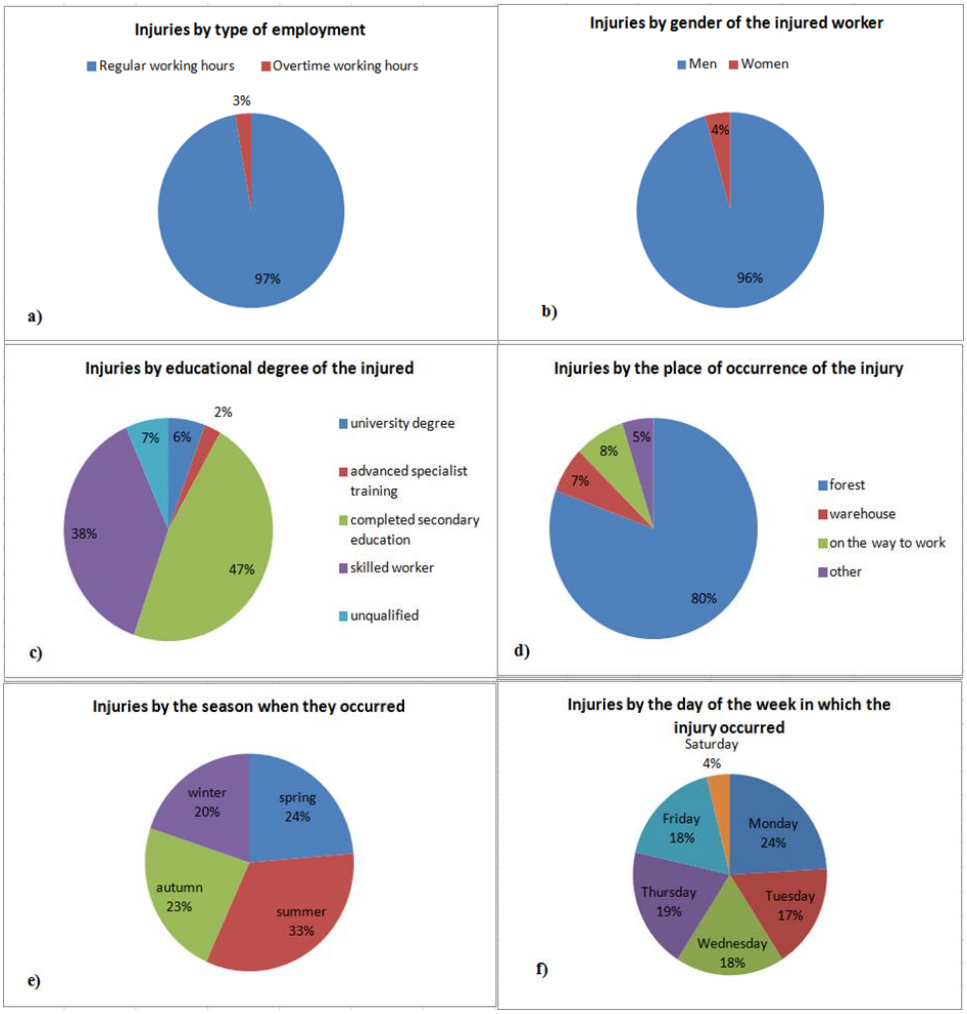


Figure 2. Etiological characteristics of occupational injuries in the Republic of Srpska forestry for the period 2011-2019

a) injuries by type of employment b) injuries by gender of the injured worker c) injuries by educational degree of the injured d) injuries by the place of occurrence of the injury e) injuries by the season when they occurred f) injuries by the day of the week in which the injury occurred

In the structure of injuries, according to the job classification (Figure 3), direct executors of woodworking operations (cutter, cutter assistant in forestry,

tractor driver, chokersetter, receiver-shipper and auxiliary worker) participate with 76% of all injuries, with the riskiest job being the cutter with 38% of all the injuries that occurred in the analysed period.

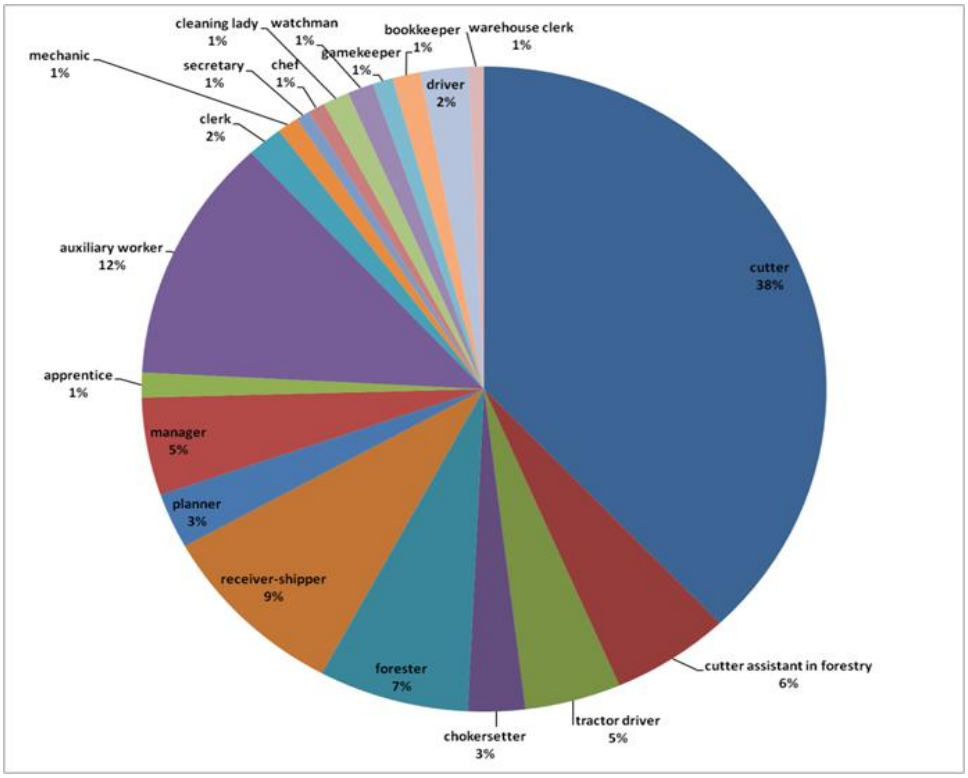


Figure 3. Structure of injuries according to the job classification in RS forestry

Of the etiological factors, from the aspect of designing occupational safety measures, the most important is to determine the source and cause of occupational injuries.

While the sources of injuries are related to material (means of labour, material, tools, etc.), energy and workplace factors that (by their direct effect on the organism of a worker) cause injuries; the causes of injuries are related to the reasons i.e. 'roots' of injuries, and they are difficult to be identified and divided into specific groups. This happens because the complex impact of multiple causes is often present. The most common causes of occupational injuries are (Vranješ, B., Tanasić, Z.,2017.):

- *subjective causes* dominated by the so-called *human factor*,
- *objective causes* related to the degree and duration of objective hazard/harm in the work environment and/or in the workplace or to the factors arising from the social environment.

The structure of occupational injuries in RS forestry by source and cause of injury is shown in Figure 4.

Based on the results of the research, we can conclude that in 1/3 of all injuries the source of the injury is the object of work-tree, and in more than 50% the sources are the object and means of work. Also, the source of injury in 19% of cases is the substrate, so more attention should be paid to the selection and purchase of adequate work shoes adapted to field working conditions.

The largest number of fatal injuries occurs in tree felling. Tree felling should be carried out professionally following the safety measures for all workers in the forest. All workers who conduct tree felling should be at an adequate distance and out of the area of risk of a falling tree.

Injuries at work mostly occur when:

- the tree falls in the wrong direction;
- the constitution of the tree causes change in the direction of the fall;
- a falling tree knocks down other trees or knocks down branches from other trees;
- there are broken branches on the felling tree, which fall off during felling and pose a threat to the worker.

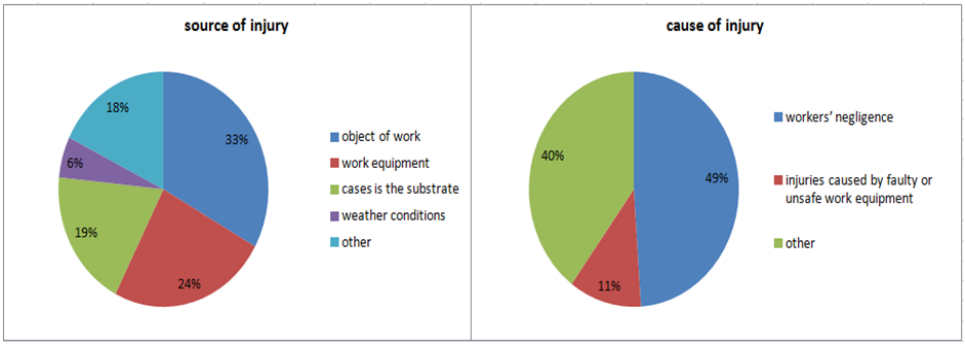


Figure 4. Sources and causes of occupational injuries in the Republic of Srpska forestry for the period 2011-2019.

While working in the forest, very dangerous operations are clearing broken trees, windthrow, damage caused by snow or sanation of the fire because of the unnatural position of trees etc. Applying technical measures for the protection of work means, safety procedures and rules for conducting work operations and training measures for workers are a good way of reducing the impact of these sources.

Theoretical sources and research data of other authors (Šporčić, M., Sabo, A. 2002). State that the most common cause of all occupational injuries is a person i.e. human error. Human error is a failure to perform a specific task, which may result in interruption of planned operations, damaging the equipment and property.

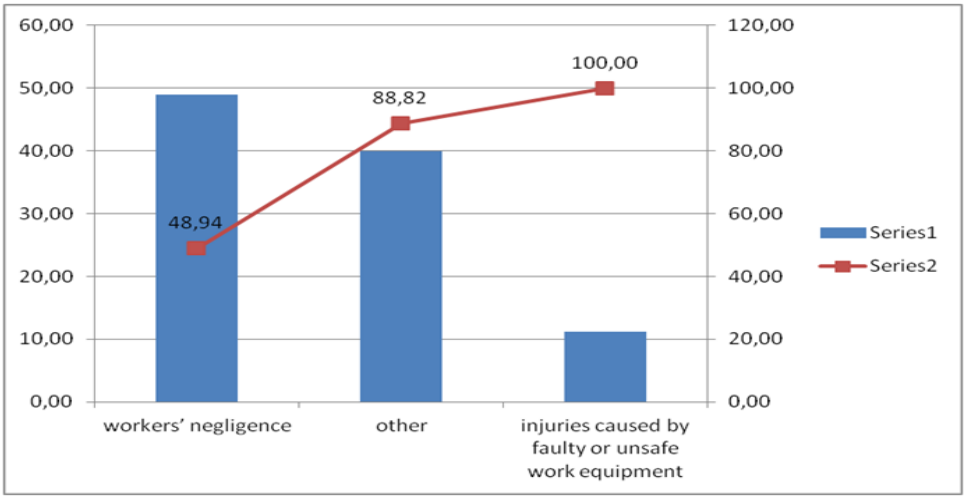


Figure 5. Pareto diagram

This error can occur due to numerous reasons: lack of precision, improper handling, lapses in recognition, cognitive and memory lapses, lapses in attention (fatigue, lack of alertness), etc. So, these are unwanted actions or activities that result in deviations from expected standard behaviours or norms, with people, equipment and system at risk (Stojiljković, E. 2020). The following figure shows the causes of occupational injuries in the Republic of Srpska forestry for the period 2011-2019 using the Pareto diagram. Based on the data obtained, we can conclude that negligence, not following safety rules, underestimation of danger, not using or misusing of personal protective equipment, etc. are the most common causes of injuries at work in forestry (e.g. in 49% of cases the cause of occupational injuries is workers' negligence). Also, according to the results obtained, we can clearly and accurately define measures of occupational safety and health at work, i.e. in what direction should we act in order to continuously reduce the risk in the workplace.

The cumulative curve of the Pareto diagram (Fig. 5) leads to the conclusion that in the system of occupational safety and health at work in forestry new forms of training and continuous education of employees for safe and healthy work and reduction of human errors should be defined, with emphasize on the significance and importance of following occupational safety measures. Considering that in 11% of all injuries that occurred the causes are the means of work, we can conclude that even technical measures for protection at work in forestry are not at an enviable level. Proper use of work means, regular inspection and maintenance of it, use of safety devices on work means, adequate use and maintenance of means and personal protective equipment etc. are some of the measures that can reduce the risk of injury at work in Republic of Srpska (BIH) forestry.

Conducting the research we found no data on occupational disease cases in this economic industry.

CONCLUSIONS

Every occupational injury causes minor or major disruption to the work processes in every industry, including forestry. For this reason and due to the fact that only a healthy, satisfied and motivated employee can express his full work potential, special attention should be paid to occupational injury analysis in every working organization.

Etiological analysis of occupational injuries in RS-BIH forestry for the period 2011-2019 shows that 76% of injuries occur during woodcutting, and that about 60% of those injuries are related to the occupation of a cutter. Also, we can see that the cause of injuries in 49% is the worker's negligence. In order to reduce the overall risk in the workplace of the company "Forests of the Republic of Srpska", it is necessary to act specifically in the area of cutting wood. It is imposed that the basic guideline for risk reduction is continuous education on safety and health at work, as well as an urgent modernization of the cutting process, that is, the automation of a cutter and auxiliary worker workplaces. By reducing the proportion of manual labour and increasing awareness about occupational safety and health, there would be a clear reduction in the number of occupational injuries. In forestry, an economic industry where workers are exposed to numerous dangers, lack of data about occupational diseases (from the aspect of occupational safety and health), is not acceptable.

Even if the occupational injury analysis shows that the by-laws do not directly affect the number and cause of injuries, it would be good to better define the measures of occupational safety and health in forestry. Also, outdated by-laws should be harmonized with other RS-BIH legislation and with modern guidelines on safety and health at work. More detailed regulation of safety and health at work of forestry workers would enable better and safer work in the field of forestry.

REFERENCES

Enez, K., Topbas, M., Acar, H.H., 2014. An evaluation of the occupational accidents among logging workers within the boundaries of Trabzon Forestry Directorate, Turkey. Int. J. Ind. Ergon. 44, 621–628.

Evropska komisija (2015). Zaštita zdravlja i sigurnosti radnika u poljoprivredi, stočarstvu,hortikulturi i šumarstvu. Luxembourg: Ured za publikacije Europske unije

Garland, J. J. (2018). Accident reporting and analysis in forestry: guidance on increasing the safety of forest work. In: Proceedings of the Forest Engineering Conference, vol. 17. Rotorua, New Zealand,.

Gerasimov, Y., Sokolov, A., 2014. Ergonomic evaluation and comparison of wood harvesting systems in Northwest Russia. Appl. Ergon. 45, 318–338.

ILO (1998). Safety and healthin forestry work. Geneva: International Labour Office.

Klun, J., Medved, M., 2007. Fatal accidents in forestry in some European countries. Croatian J. For. Eng. 28, 55–63.

Kogler, R., Quendler, E., Boxberger, J., 2015. Analysis of occupational accidents with agricultural machinery in the period 2008–2010 in Austria. Saf. Sci. 72, 319–328.

Krstić I., Anđelković B. (2013). Profesionalni rizik. Niš: Univerzitet u Nišu, Fakultet zaštite na radu u Nišu.

Landekić, M. (2010). Organizacijska kultura i sigurnost pri radu u Hrvatskom šumarskom sektoru, Šumarski list br. 11–12, CXXXIV (2010), str. 613-622

Lilley, R., Feyer, A.M., Kirk, P., Gander, P., 2002. A survey of forest workers in New Zealand – Do hours of work, rest, and recovery play a role in accidents and injury? J. Saf. Res. 33, 53–71.

Lindroos, O., Burstrom, L., 2010. Accident rates and types among self-employed private forest owners. Accid. Anal. Prev. 42, 1729–1735.

Milijić, V. (2013). Zaštita na radu u šumi. Majdanpek: Resurs centar Majdanpek

Musić, J., Halilović, V., Lojo, A., Šporčić, M., Đonlagić, A. (2019). Analiza sigurnosti pri radu u šumarstvu Federacije BiH – studija slučaja. Nova mehanizacija šumarstva: časopis za teoriju i praksu šumarskoga inženjerstva, 40 (2019), Zagreb, https://doi.org/10.5552/nms.2019.4

Spasić, D .(2001). Ekonomika zaštite na radu. Niš: Grafičko preduzeće SVEN.

Stojiljković, E. (2020). Procena ljudske pouzdanosti. Monografija nacionalnog značaja. Niš: Univerzitet u Nišu, Fakultet zaštite na radu u Nišu.

Sullman M. J. M, P M. Kirk, R. Parker, J. E. Gaskin . 1999 **,** New Zealand Logging Industry Accident Reporting Scheme: Focus for a Human Factors Research Programme

Šporčić, M., Sabo, A. (2002). Ozlijeđivanje radnika u hrvatskom šumarstvu tijekom razdoblja 1991-2000, Šumarski list br. 5–6, CXXVI (2002), str. 261-271

Thelin, A., 2002. Fatal accidents in Swedish farming and forestry, 1988–1997. Saf. Sci. 40, 501–517.

Vranješ, B., Tanasić, Z. (2017). The application of the Ishikawa method for determining the cause of accidents at work, 13th International Conference on Accomplishments in Mechanical and Industrial Engineering DEMI 2017, Banja Luka 26th - 27th May 2017, pp. 827-834.

Yoshimura, T., Acar, H.H., 2004. Occupational safety and health conditions of forestry workers in Turkey. J. For. Res. 9, 225–232.

Savosko, V., Tovstolyak, N., Lykholat, Y., Grygoryuk, I. (2020): Structure and diversity of urban park stands at Kryvyi Rih ore-mining & metallurgical district, central Ukraine. Agriculture and Forestry, 66 (3): 105-126.

DOI: 10.17707/AgricultForest.66.3.10

Vasyl SAVOSKO, Natalia TOVSTOLYAK, Yuryi LYKHOLAT, Ivan GRYGORYUK [1]

STRUCTURE AND DIVERSITY OF URBAN PARK STANDS AT KRYVYI RIH ORE-MINING & METALLURGICAL DISTRICT, CENTRAL UKRAINE

SUMMARY

The present study examines the relationships between structure (floristic composition, dendrometric parameters), diversity (diversity and evenness indexes) of urban forest park stands and the ecological (soil fertility, soil moisture), environmental factors (air pollution). The study is based on the forest park stands inventory data, performed from 2012 to 2017 in Kryvyi Rih City, Central Ukraine. The floristic compositions of the urban forest park stands are poor. There are only 23 species that belong to 14 families and 12 genera. More families were represented by at least more than 2% of taxon diversity. While Ulmaceae (2 genera, 4 species–17,39 %), Fabaceae (3 genera, 2 species–17,39 %), Aceraceae (1 genera, 4 species–17,39 %) were the most representative families. It was established that at forest park the values of stand density varied from 490 to 660 trees*ha^{-1}, stem heights were from 26 to 31 m, stem diameters were from 13 to 17 cm, stand basal area were from 32 to 49 m^2*ha^{-1}, stand volume were from 200 to 415 m^3*ha^{-1}. the values of relative stem heights were from 0,63 to 0,82 m*year^{-1}, relative stem diameters were from 0,31 to 0,43 cm*year^{-1}, relative stand basal area were from 0,80 to 1,19 m^2*ha^{-1}*year^{-1}, relative stand volume were from 5,45 to 10,28 m^3*ha^{-1}*year^{-1}. The varied values of the forest park stands index (Shannon-Wiener diversity index from 0,75 to 1,61, Pielou's evenness index from 0,53 to 0,86, Simpson's diversity from 0,24 to 0,60, Margalef's diversity index from 0,87 to 6,97) indicate the ecological instability of these woody plant communities. Current state of the urban forest park stands determined by the combined influence of ecological (soil fertility, soil moisture) and environmental factors (air pollution).

Keywords: Floristic Composition, Dendrometric Parameters, Stand Basal Area, Stand Volume, Diversity and Evenness Index.

[1]Vasyl Savosko (corresponding author: savosko1970@gmail.com), Natalia Tovstolyak, Kryvyi Rih State Pedagogical University, Kryvyi Rih, UKRAINE; Yuryi Lykholat, Oles Honchar Dnipro National University, Dnipro, UKRAINE; Ivan Grygoryuk, National University of Life and Environmental Sciences of Ukraine, Kyiv, UKRAINE

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:25/02/2020 *Accepted:20/07/2020*

INTRODUCTION

Unfortunately, exploding human populations, 6 billion people are degrading the environment at an accelerating rate, especially in developing and undeveloped countries (Janhäll, 2015; Kim, 2016; Verma and Raghubanshi, 2018). Besides, anthropogenic activities have triggered unprecedented environmental changes on a global and on a local scale. Especially anthropogenic activities strictly available in metallurgical and mining areas (Carvalho, 2017; Urošević et al 2018) such as Kryvyi Rih basin (Bielyk et al 2020; Savosko et al., 2019a; Savosko et al., 2020).

It should also be noted that, due to increasing congested cities all over the world, people's well-being and people's health are at stake. That's why, over the past few decades, there have been intensive efforts to "green cities", reflected in a surge of interest in innovations such as "green walls" (Janhäll S., 2015; Nowak et al, 2018; Rode et al, 2016). Therefore, it is so important to ensure the sustainable existence of the urban forest park stands.

As known urban forest park stands have a great importance in terms of nature and landscape protection. They represent a natural habitat for a wide range of other organisms, serve public welfare, and contribute to popularization, planting and maintenance of plants. Besides, they play an important role in improving various environmental factors in urban areas, such as: temperature, humidity, and dust/pollution content. They also provide sustainable development of the city, either through local climate regulation, carbon sequestration or reduction of stormwater runoff (Anguluri and Narayana, 2017; Rode et al, 2016). Generally, urban forest park stands which are integral to human health represent a complex and necessary feature of the urban landscape, as well as areas provide ecosystem services.

In recent decades, ecosystem services is widely used among scientists and policy makers to highlight the importance of the environment in sustaining human livelihoods in industrial areas (Olander et al, 2018; Rode et al, 2016), especially in metallurgical and mining basins such as Kryvyi Rih City, Central Ukraine (Savosko and Tovstolyak, 2017; Savosko et al, 2019a).

Ecosystem services are generally defined as goods and services that are of value to people, provided wholly or in part by ecosystems services including and urban forest park stands (Lykholat et al, 2016a; Song et al 2018). These three attributes are as well defining ecosystems services of the urban forest park stands: 1) floristic composition, 2) dendrometric characteristics, 3) diversity and evenness indices (Booth 2018; Ebrahimigajoti et al, 2013; Kolbe et al, 2016; Lykholat et al, 2016b; Liquete et al 2016). Floral composition is the oldest and most common way of assessing the structure of the forest stands. The dendrometric characteristics of trees at stands are usually evaluated using a set of variables such as diameter at breast height, tree height, crown projection area, crown length and height of the crown base. Species and biological diversity have always been some of the most frequently used evaluation measures for forest

communities in the general forestical monitoring work (Chivulescu et al, 2018; Solomou and Skoufogianni, 2016).

Numerous studies have analyzed the modern state of the urban forest park stands (Verma and Raghubanshi, 2018; Voigt et al, 2014). Nevertheless, these studies are limited by the lack of research on one attribute (or floristic composition, ore dendrometric characteristics, ore diversity and evenness indices). Moreover, the estimates of forest park stands in urban environments mainly rely upon methodologies developed for trees in traditional forests. More exact quantification of urban forest park stands may depend on development of comprehensive research especially at different habitats.

It is widely accepted that understanding conservation targets and sustainable development of the urban forest park stands, including a full description of woody species floristic composition, dendrometric parameters and diversity and evenness indexes (Martins and Pereira, 2018). Furthermore, description and analysis of urban forest park stands through floristic and dendrometric, diversity and evenness characterization provide means to analyse the relation between woody vegetation and edaphic, topographic and human factors (Dan et al, 2016; Lykholat et al 2019a; Lykholat et al 2019b).

The main objectives of this work were to (i) analyze of urban forest park stands floristic composition in the Kryvyi Rih City (Central Ukraine), (ii) estimate their dendrometric parameters and (iii) assess their diversity and evenness indexes. As no detailed ecological information is available on the urban forest park stands, it was not possible to set ecological hypotheses. However, assuming that the pattern of woody species and stands types results from different stochastical events, it was hypothesized that current state of the urban forest park stands determined by the combined influence of ecological (soil fertility, soil moisture) and environmental factors (air pollution).

MATERIALS AND METHODS

Study area.

This study was conducted in the forest park woodlands, which is located in the Kryvyi Rih City (Ore-mining and metallurgical basin, Central Ukraine). The study area is located between 47°53'54" and 48°8'52" north latitude and 33°19'52" and 33°33'38" west longitude (Figure 1). The climate of the study area is characterized by a short spring, dry summer, little snowy cold winters. The air temperatures average–3,5 and 21,8°C in January and July, respectively. Precipitation falls primarily as rain averaging 400-450 mm $year^{-1}$.

Research design and data collection.

During 2012-2017 we performed a comprehensive study of Kryvyi Rih District parks: 1) studied the history of parks, 2) analyzed the evolution of parks (their progress / regress), 3) studied the ecological conditions in parks, 4) studied the floristic composition of all plant trees in parks, 5) identified treys plantations similar to forest (woody stands, park stands), 6) studied of the structure and diversity of urban park stands.

Map of Ukraine

Map of Kryvyi Rih City

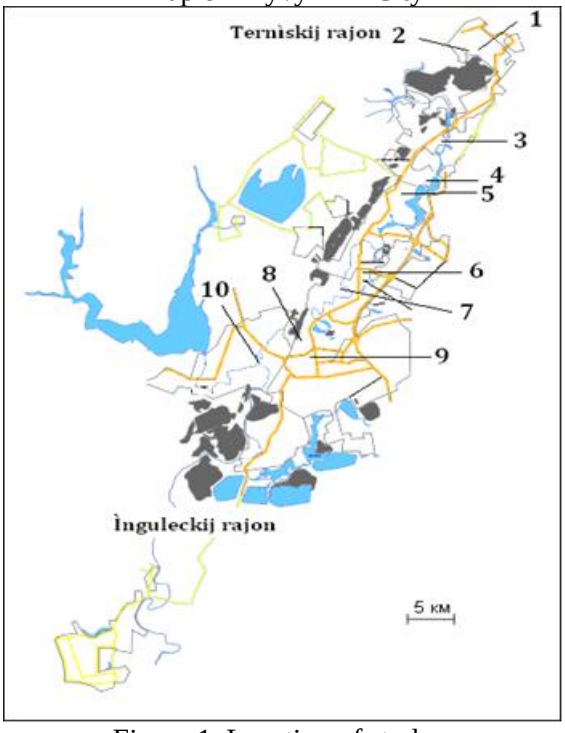


Figure 1. Location of study areas

1 – Pivnichnyi Park, 2 – Pershoho Travnia Mine's Park, 3 – Ternivskyi Park,
4 – Shakhtarskyi Park, 5 – Zatyshok Park, 6 – Cinema Iunist's Park,
7 – Palace of Culture of Mine's Rodina Park, 8 – Rudanivskyi Park,
9 – Bohdan Khmelnytskyi's Park, 10 – Fedor Mershavtsev's Park

Therefore, we choose only ten city parks for the purpose of this study: Pivnichnyi Park, Pershoho Travnia Mine's Park, Ternivskyi Park, Shakhtarskyi Park, Zatyshok Park, Cinema Iunist's Park, Palace of Culture of Mine's Rodina Park, Rudanivskyi Park, Bohdan Khmelnytskyi's Park, Fedor Mershavtsev's Park (Figure 1).

Each of these parks combining a contrasting ecological & environmental condition of their areas, diverse floristic composition and have forest woodlands. Summing up of the soil factors actions (fertility and moisture) and of the air pollution actions three ecological areas were identified: 1) environmentally friendly area (Background), 2) relative environmentally friendly area (Buffer), 3) relative environmentally not friendly area (Impact) (Savosko et al, 2019b).

A forest park woodland inventory was made a random sampling scheme. During 2012-2017, the 22 research plots (20*20 m) were established in the park's ecosystems: from 1 plot to 5 plots per park. Field data were collected through direct enumeration and measurement of all trees in every plot. In each plot, all woody stems of diameter at breast height (dbh) > 10 cm were recorded and: 1) their diameter at 1,3 m above ground (in two perpendicular directions by a caliper); 2) their height (by a hypsometer) were measured (West, 2009). The binomial nomenclature of each tree species was recorded following the Modern botanical nomenclature (IPNI, 2020) as a well as the number of trees per species.

Dendrometric parameters

For each research plot the following dendrometric parameters were computed: tree-density of the stand (N), i.e. the average number of trees per sample plot, expressed as trees per hectare (West, 2009).

For each tree the following absolute dendrometric parameters were computed:

– *basal area of the tree* (Gabs), i.e. the cross-sectional area at 1.3 m above ground level of tree, expressed as (m^2):

$$Gabs = \frac{\pi * D * 2}{40000} \quad (1)$$

where, π is constant (3,142), d is the diameter (in cm) of the tree;

– *volume of the tree* (Vabs), expressed as (m^3):

$$Vabs = K * Gabs * H \quad (2)$$

where, K is constant (0,415), Gabs is basal area of the tree (m^2), H is the height of the tree (m).

For each tree the relative dendrometric parameters were computed using the following formula:

- $$Hrel = \frac{Habs}{N} \quad (3)$$

where, Hrel is relative height of the tree ($m*year^{-1}$), Habs is absolute height of the tree (m), N is stand age (year);

– $Drel = \frac{Dabs}{N}$ (4)

where, Drel is relative diameter of the tree (cm*year^{-1}), Dabs is absolute diamete of the tree (m), N is stand age (year);

– $Grel = \frac{Gabs}{N}$ (5)

where, Grel is relative d basal area of the tree (m^2*year^{-1}), Gabs is absolute basal area of the tree (m^2), N is stand age (year);

– $Vrel = \frac{Vabs}{N}$ (6)

where Vrel is relative volume of the tree (m^3*year^{-1}), Vabs is absolute volume of the tree (m^2), N is stand age (year).

Diversity and evenness indexes calculation

The Shannon-Wiener's diversity index, Pielou's evenness index, Simpson's diversity index and Margalef's richness index was used in order to study.

The Shannon-Wiener's diversity index (H') is defined by (Shannon and Wiener, 1949):

- $H' = -\sum_{i=1}^{S} pi * \ln pi$ (7)

where, H' – Shannon-Wiener's diversity index, S – number of species, pi – proportion of total sample belonging to the *i*th species, ln – natural logarithm.

The Pielou's evenness index (Eq) is defined by (Pielou, 1966):

- $Eq = \frac{H'}{\ln(S)}$ (8)

where, Eq – Pielou's evenness index, H' – Shannon-Wiener's diversity index, S – number of species.

The Simpson's diversity index (E) is defined by (Simpson, 1949):

- $E = \sum_{i=1}^{S} (pi)^2$ (9)

where, E – Simpson's diversity index, S – number of species, pi – proportion of total sample belonging to the *i*th species.

The Margalef's richness index (Ma) is defined by (Margalef, 1958):

- $Ma = \frac{(S-1)}{Ln(N)}$ (10)

where, Ma – Margalef's richness index, S – number of species, N – total number of individuals.

For diversity and evenness indexes calculated data of forest park woodlands stand density, stand volume and stand basal area were used (Kaufman et al, 2017; Spake and Doncaster, 2017).

Statistical analysis

The data were submitted to descriptive statistics and analysis of variance (ANOVA). The statistical analysis was performed using the program SPSS for Windows, version 11.0.0. For all statistical analysis, significance was considered $P < 0,05$ (McDonald, 2014).

RESULTS

Characteristics of the parks

At Kryvyi Rih ore-mining & metallurgical district city parks (in the modern sense) began to be created in the late XIX and early XX centuries, simultaneously with "iron fever". However, these parks were small and in fact they have not survived to this day. In the 20's and 30's of the twentieth century, the rapid industrialization of the former USSR led to the powerful development of the Kryvyi Rih City and surrounding mining villages. At this time, the erection of industrial enterprises and the creation of numerous city gardens and parks were begun. The first City Parks, which include massive stands, were establishment in the 20-30s of the twentieth century (Table 1).

It was during this time stands formation was implemented in the following parks: Fedor Mershavtsev's Park (1929), Rudanivskyi Park (1930), Bohdan Khmelnytskyi's Park (1935) and Palace of Culture of Rodina Mine's Park (1938). However, during World War II, their stands were severely damaged. In the post-war years, the reconstruction of old parks and the creation of new parks (mostly between 1945 and 1965) were made. Among the studied parks of this region, the stand of Cinema Iunist's Park (established in 1975) are the youngest.

In most cases, the City Parks at Kryvyi Rih District are located on Interfluve plateau the so-called plakor (Table 1). In these areas, zonal climatic and soil conditions are best manifested. In addition to the interfluve plateau among the landforms and surface of City Parks it was also found seepage slope and midslope. In these areas, very extreme soil conditions are manifested. On the territory of two parks (Ternivskyi Park and Shakhtarskyi Park) among the landforms floodplain of small stream are also deployed. In these areas, good soil conditions are manifested. Only one park (Fedor Mershavtsev's Park) is located on Foodplain, with alluvial toeslope. In these areas, very good soil conditions are manifested.

At Kryvyi Rih District soils were classified as Chernozems by International Soil Classification Systems (SCS) (World reference base for soil resources, 2014), Chernozems Ordinary by Ukrainian SCS (Polupan et al 2005) and Mollisols by USDA SCS (Soil Survey Staff, 2014).

Table 1. The main characteristics of the parks at Kryvyi Rih District

Park	Year of establishment	Area, ha	Ecological factors				Environ-mental factors, levels of the air pollution
			Landforms and surface	Soil			
				Texture	Moisture	Fertility	
Pivnichnyi Park	1963	27,0	Interfluve plateau, seepage slope, midslope	Clay loam	Dry, slightly moist, moist	Good fertility	Moderate
Pershoho Travnia Mine's Park	1949	8,0	Interfluve plateau, seepage slope	Clay loam	Dry, slightly moist	Good fertility	Moderate
Ternivskyi Park	1963	7,7	Interfluve plateau, seepage slope, midslope, floodplain of small stream	Clay loam	Dry, slightly moist, moist	Good fertility	Low
Shakhtarskyi Park	1950	42,8	Interfluve plateau, seepage slope, midslope, floodplain of small stream	Clay loam	Dry, slightly moist, moist	Good fertility	Low
Zatyshok Park	1962	3,6	Interfluve plateau	Clay loam	Dry, slightly moist	Good fertility	Low
Cinema Iunist's Park	1975	3,5	Interfluve plateau,	Clay loam	Dry, slightly moist	Good fertility	Low
Palace of Culture of Rodina Mine's Park	1938	4,9	Interfluve plateau	Clay loam	Dry, slightly moist	Good fertility	Moderate
Rudanivskyi Park	1930	14,0	Midslope, colluvial footslope	Clay loam	Slightly moist	Good fertility	Moderate
Bohdan Khmelnytskyi's Park	1935	42,0	Seepage slope, midslope	Clay loam	Dry, slightly moist	Good fertility	High
Fedor Mershavtsev's Park	1929	36,0	Foodplain, alluvial toeslope	Sandy clay loam	Moist	Very good fertility	Very low

Soil texture (such as loam, sandy loam or clay) as known, refers to the proportion of sand, silt and clay sized particles that make up the mineral fraction of the soil. In its turn, soil texture significantly effects on soil fertility and on the growth / development of woody plant species. The results of our research showed that in City Park at Kryvyi Rih District, the soil texture was represented by the following classes. The first class, the clay loam soil, was found in most parks. The second class, sandy clay loam soil was found in only one park (Fedor Mershavtsev's Park). Generally, soil texture in City Parks at Kryvyi Rih District is favorable for woody plants. As we noted earlier, Kryvyi Rih District is located in arid areas. Therefore, moisture status of soil is very relevant for our research of the City Parks. We found that in most cases soil moisture can be estimated as dry-slightly moist. In some cases (Ternivskyi Park and Shakhtarskyi Park), soil moisture can be estimated as slightly moist-moist. Only in one case soil moisture can be estimated as Slightly-moist (Rudanivskyi Park) and moist (Fedor Mershavtsev's Park). Generally, soil moisture in City Parks at Kryvyi Rih District is a few and a slightly favorable for woody plants. Therefore, in the future it is very important to reconstruct old irrigation systems and create new irrigation systems. The soils of the Kryvyi Rih District (Chernozems by Internutional SCS and Mollisols by USDA SCS) are some of the most fertile soils in the World. That is why the soil fertility of City parks can only be assessed as "Good fertility" (in most cases) and "Very good fertility" (Fedor Mershavtsev's Park).

At Kryvyi Rih District, the presence of a significant number of powerful mining and metallurgical enterprises causes significant air pollution, which worsens the ecological conditions for the growth of tree species. According to the common classification scheme, there are the following levels of air pollution: 1) very low, 2) low, 3) moderate and 4) high. For very low level of air pollution the following concentrations are available: Carbon monoxide (CO) from 0,600 $mg*m^3$ to 1,500 $mg*m^3$; Nitrogen dioxide (NO_2) from 0,008 $mg*m^3$ to 0,020 $mg*m^3$; Sulfur dioxide (SO_2) from 0,010 $mg*m^3$ to 0,025 $mg*m^3$; Dust from 0,030 $mg*m^3$ to 0,075 $mg*m^3$. For low level of air pollution the following concentrations are available: Carbon monoxide (CO) from 1,500 $mg*m^3$ to 3,000 $mg*m^3$; Nitrogen dioxide (NO_2) from 0,020 $mg*m^3$ to 0,040 $mg*m^3$; Sulfur dioxide (SO_2) from 0,025 $mg*m^3$ to 0,050 $mg*m^3$; Dust from 0,075 $mg*m^3$ to 0,150 $mg*m^3$. For moderate level of air pollution the following concentrations are available: Carbon monoxide (CO) from 3,000 $mg*m^3$ to 6,000 $mg*m^3$; Nitrogen dioxide (NO_2) from 0,040 $mg*m^3$ to 0,080 $mg*m^3$; Sulfur dioxide (SO_2) from 0,050 $mg*m^3$ to 0,100 $mg*m^3$; Dust from 0,150 $mg*m^3$ to 0,300 $mg*m^3$. For moderate level of air pollution the following concentrations are available: Carbon monoxide (CO) from 6,000 $mg*m^3$ to 12,000 $mg*m^3$; Nitrogen dioxide (NO_2) from 0,080 $mg*m^3$ to 0,160 $mg*m^3$; Sulfur dioxide (SO_2) from 0,100 $mg*m^3$ to 0,200 $mg*m^3$; Dust from 0,300 $mg*m^3$ to 0,600 $mg*m^3$. Generally, the very low level of air pollution is typical for the area of one City Park, the low level of air pollution is typical for the area of for City Parks, the moderate level of air

pollution is typical for the area of for City Parks and the High level of air pollution is typical for the area of one City Park (Table 1).

Floristic composition

Table 2. Occurrence of the woody plant species in parks at Kryvyi Rih District

Scientific name	Common name	In parks		In research plots	
		Np	%	Nsp	%
Acer negundo L.	Ashleaf maple	3	30	3	13,64
Acer pseudoplatanus L	Sycamore maple	2	20	2	9,09
Acer saccharinum L	Silver maple	1	10	1	4,55
Acer platanoides L.	Norway maple	10	100	15	68,18
Aesculus hippocastanum L.	Horse chestnut	6	60	6	27,27
Celtis occidentalis L.	Common hackberry	1	10	1	4,55
Cotinus coggygria Scop.	European smoketree	1	10	1	4,55
Crataegus × *kyrtostyla* Fingerh. (known in Ukraine as *Crataegus fallacina* Klokov)	Oneseed hawthorn	1	10	2	9,09
Fraxinus americana L.	White ash	2	20	2	9,09
Fraxinus excelsior L.	European ash	5	50	7	31,82
Gleditsia triacanthos L.	Honey locust	2	20	4	18,18
Juglans regia L.	English walnut	1	10	1	4,55
Morus nigra L.	Black mulberry	4	40	4	18,18
Populus nigra L.	Black cottonwood	1	10	1	4,55
Quercus robur L.	English oak	5	50	7	31,82
Robinia pseudoacacia L.	Black locust	4	40	7	31,82
Robinia viscosa Michx. ex Vent.	Clammy locust	1	10	1	4,55
Styphnolobium japonicum (L.) Schott	Japanese pagoda tree	1	10	1	4,55
Tilia cordata Mill.	Littleleaf linden	4	40	5	22,73
Tilia platyphyllos Scop.	Largeleaf linden	3	30	4	18,18
Ulmus glabra Huds.	Mountain elm	8	80	9	40,91
Ulmus laevis Pall.	European white elm	2	20	4	18,18
Ulmus minor Mill.	European field elm	6	60	7	31,82

Np – total number of parks, Nsp – total number of research plots, % – frequency of occurrence.

According to our research (Savosko and Tovstolyak 2017; Savosko et al., 2018; Savosko et al., 2019b) and data other authors (Fedorovskiy et al., 2013; Terlyga et al., 2015), more than 150 species of trees and shrubs grow in parks, but, in park sand only 23 tree plant species was found. These species belonged to 14 families and 12 genera. More families were represented by at least more than 2% of taxon diversity. While Ulmaceae (2 genera, 4 species – 17,39 %), Fabaceae (3 genera, 2 species – 17,39 %), Aceraceae (1 genera, 4 species – 17,39 %) were the most representative families (Table 2 and Figure 2).

The tree plant species has different incidence among research plots in forest park stand at Kryvyi Rih City. Such, Norway maple (*Acer platanoides* L.) was distributed in 15 plots (65,22 %). Mountain elm (*Ulmus glabra* Huds.) was distributed in 9 plots (39,13 %). Four tree plant species were distributed in 7 plots(by 30,43 %): Black locust (*Robinia pseudoacacia* L.), English elm (*Ulmus minor* Mill.), English oak (*Quercus robur* L.), European ash (*Fraxinus excelsior* L.). Horse chestnut (*Aesculus hippocastanum* L.) was distributed in 6 plots (20,69 %). Littleleaf linden (*Tilia cordata* Mill.) was d4stributed in 5 plots (21,74 %). Other 14 tree plant species were distributed only in 1-4 research plots in forest park stand at Kryvyi Rih City. The forest park stand consisted of a small number of tree plant species. Such, 6 research plots (27,27 %) had 2 species, 5 research plots (22,73 %) had 6 species, 4 research plots (18,18 %) had 5 species, 3 research plots (13,64 %) had 3 species. The maximum number of species (7 and 8) was found only in 1 and 2 plots, respectively. It should also be noted that only one species of trees grew on 1 plot.

Dendrometric parameters

The results of the statistical analysis and Student test of the absolute dendrometric parameters forest park stand are presented in Table 3. It was established that at Kryvyi Rih forest park the values of stand density varied from 490 to 660 trees $*ha^{-1}$, stem heights were from 26 to 31 m, stem diameters were from 13 to 17 cm, stand basal area were from 32 to 49 $m^{2*}ha^{-1}$, stand volume were from 200 to 415 $m^{3}*ha^{-1}$. The coefficients of variation of absolute dendrometric parameters were from 15,08 % to 32,19 %, which, according to the criteria established by Pimentel-Gomes and Garcia (2002), indicate medium and too high variability (Pimentel-Gomes and Garcia, 2002).

The values of stand density were significantly differed only between two ecological areas: Impact and Background ($P < 0,05$). In contrast, differences among values of the mean height were found to be not significant ($P > 0,05$). The values of Diameter, Basal area, Volume were significantly differed between all ecological areas: Buffer and Background ($P < 0,001$), Impact and Background, ($P < 0,001$). In compared with Background ecological areas at Buffer ecological areas values of absolute dendrometric parameters of the forest park stand were higher: stem diameters by 10,34 %, stand basal area by 16,41 %, stand volume by

8,77 %. In compared with Background ecological areas at Impact ecological areas values of absolute dendrometric parameters of the forest park stand were less: stem diameters by 11,79 %, stand basal area by 11,05 %, stand volume by 54,79 %.

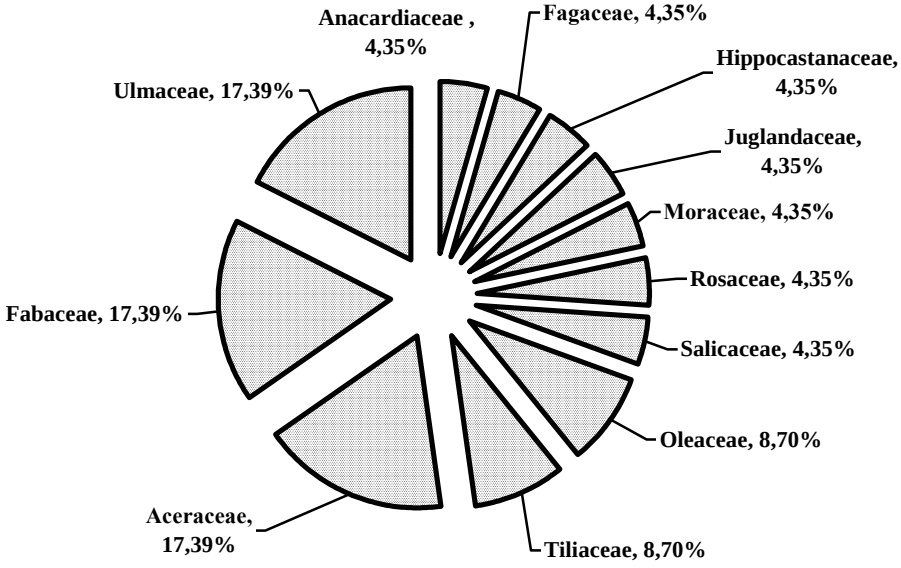


Figure 2. Floristic composition of the forest stands in parks at Kryvyi Rih District

Table 3. The absolute dendrometric parameters of the forest stand in parks at Kryvyi Rih District.

Ecological areas	Statistical Index	Density (N), trees/ha	Height, m (Habs)	Diameter, cm (Dabs)	Basal area, m^2/ha (Gabs)	Volume, m^3/ha (Vabs)
Background, n=4	Mean	528	28,3	14,70	38,3	361
	Std.	18	0,4	0,14	1,0	11,0
	CV, %	27,32	21,45	29,96	26,74	29,61
Buffer, n=11	Mean	626*	29,5	16,22**	44,6*	393*
	Std.	16	0,5	0,18	2,1	10,9
	CV, %	20,62	15,08	26,09	25,78	26,12
Impact, n=7	Mean	542	26,78	13,15**	34,5*	233**
	Std.	19	0,6	0,16	1,5	15,8
	CV, %	23,81	16,63	32,19	29,81	23,98

n – numbers of plots, Mean – arithmetic mean, Std. – standard deviation of mean, CV, % – coefficient of variation; * – are significantly different between Background and another Ecological areas at probability less then 0,05 (P <0,05);** – are significantly different between Background and another Ecological areas at probability less then 0,01 (P <0,01).

As it is known, the forest park stands at Kryvyi Rih City have different ages. Therefore, comparing their absolute dendrometric parameters is not correct. Therefore, we calculated and analyzed the relative dendrometric parameters (Table 4). The findings of this study indicated that at Kryvyi Rih forest park the values of relative stem heights were from 0,63 to 0,82 $m*year^{-1}$, relative stem diameters were from 0,31 to 0,43 $cm*year^{-1}$, relative stand basal area were from 0,80 to 1,19 $m^{2*}ha^{-1}$ $*year^{-1}$, relative stand volume were from 5,45 to 10,28 m^3*ha^{-1} $*year^{-1}$.

Table 4. The relative dendrometric parameters of the forest stand in parks at Kryvyi Rih District

Ecological areas	Statistical Index	Height, $m*year^{-1}$ (Hrel)	Diameter, $cm*ear^{-1}$ (Drel)	Basal area, $m^2/ha*ear^{-1}$ (Grel)	Volume, $m^3/ha*ear^{-1}$ (Vrel)
Background, n=4	Mean	0,71	0,37	0,96	9,02
	Std.	0,02	0,01	0,03	0,21
	CV, %	11,17	12,78	23,07	22,71
Buffer, n=11	Mean	0,74	0,41**	1,11**	9,82*
	Std.	0,04	0,01	0,04	0,23
	CV, %	15,08	16,09	20,78**	16,12
Impact, n=7	Mean	0,67	0,33**	0,86	5,83***
	Std.	0,02	0,01	0,03	0,19
	CV, %	16,63	22,19	29,8	33,98

n – numbers of plots, Mean – arithmetic mean, Std. – standard deviation of mean, CV, % – coefficient of variation, * – are significantly different between Background and another Ecological areas at probability less then 0,05 (P <0,05), ** – are significantly different between Background and another Ecological areas at probability less then 0,01 (P <0,01), *** – are significantly different between Background and another Ecological areas at probability less then 0,001 (P <0,001).

The coefficient of variation (CV) was used to evaluate the variability of the data. According to the criterion proposed by Pimentel-Gomes F, Garcia CH. (2002), the CVs were classified as follows: medium (10% <CV≤ 20%) for relative stem heights at all ecological areas, relative stem diameters at Background and Buffer areas relative stand volume at Buffer area; high (20% <CV≤ 30%) for relative stem diameters at Impact area, relative stand basal area at all ecological areas, relative stand volume at Background area and very high (CV> 30%) only for relative stand volume at Impact area.

It was found that differences among values of relative stem heights were not significant (P> 0,05) between Background ecological area (with one hand) and Buffer/Impact ecological areas (on the other hand). In contrast, the values of relative stem diameters, relative stand basal, relative stand volume were significantly differed between all ecological areas: Background and Buffer (P < 0,001), Background and Impact (P < 0,001). In compared with Background

ecological areas at Buffer ecological areas values of relative *dendrometric* parameters of the forest park stand were higher: stem diameters by 10,81 %, stand basal area by 15,63 %, stand volume by 8,77 %. In compared with Background ecological areas at Impact ecological areas values of absolute *dendrometric* parameters of the forest park stand were less: stem diameters by 10,81 %, stand basal area by 10,42 %, stand volume by 35,37 %.

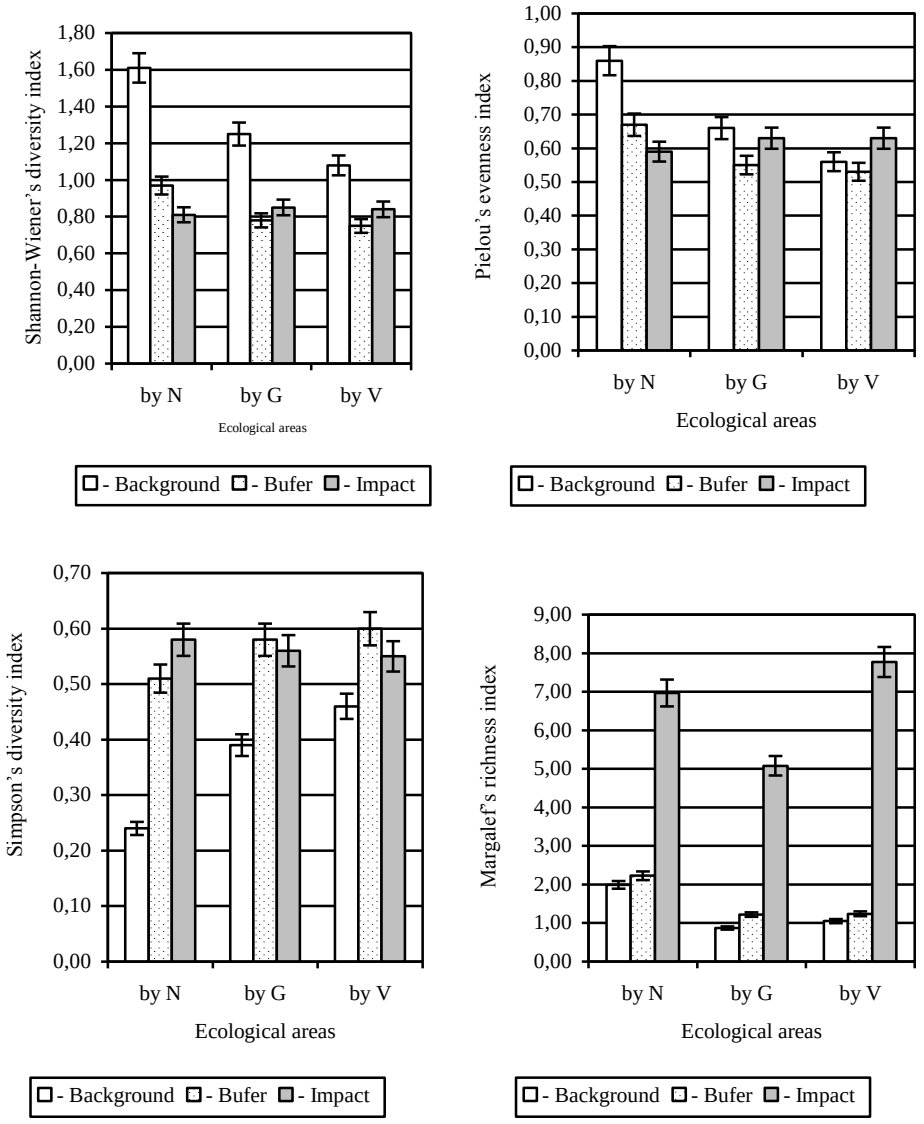


Figure 3. Diversity and evenness indexes of the forest park stand at Kryvyi Rih City, Central Ukraine

N – stem density, V – stem volume, G – stem basal area

Diversity and evenness indexes

Was found that at Kryvyi Rih City forest park stand the Shannon-Wiener diversity index values varied from 0,81 to 1,61 (calculated by stem density), varied from 0,75 to 1,08 (calculated by stand volume) and varied from 0,78 to 1,25 (calculated by stand basal area). For this index calculated by stem density a clear downward trend in ecological areas was installed (Figure 3).

It is important to note that in Background ecological area the maximum values of Shannon-Wiener index were established. The data Figure 3 show that the Pielou's evenness index values varied from 0,59 to 0,86 (calculated by stem density), varied from 0,53 to 0,56 (calculated by stand volume) and varied from 0,55 to 0,66 (calculated by stand basal area). As in the previous case, the maximum values of this index in Background area also were revealed. The results of our calculations showed that the Simpson's diversity index values varied from 0,24 to 0,58 (calculated by stem density), varied from 0,46 to 0,60 (calculated by stand volume) and varied from 0,39 to 0,58 (calculated by stand basal area). According to our research (Figure 3) the Margalef's diversity index values varied from 0,87 to 1,99 (calculated by stem density), varied from 1,22 to 2,23 (calculated by stand volume) and varied from 5,08 to 6,97 (calculated by stand basal area). In all cases, in the Background ecological area the values of this index were maximal.

DISCUSSION

This paper provides information on forest park stands at Kryvyi Rih City, which are in steppe and technogenesis conditions (a case study at Ukraine). Until now, relatively little information was available on floristic compositions, dendrometric characteristics and diversity / evenness indexes of forest park stands in this region.

At Kryvyi Rih City and at Kryvyi Rih Region there are enough trees and shrubs of the species which had been naturalized in steppe and technogenesis conditions. Such, in Kryvyi Rih Botanic Garden Arboretum about 1000 trees and shrubs species and cultivars grow (Fedorovskiy and Mazur, 2007). In the flora of the Dnipropetrovsk oblast, more than 150 trees and shrubs species are identified (Tarasov, 2012). In Kryvyi Rih City gardens, parks and squares there are about 150 trees and shrubs species (Savosko and Tovstolyak, 2017). It was also established that 80 trees and shrubs species naturally grow on devastating lands at this region (Savosko et al, 2019b). But for green building of the forest park stand at Kryvyi Rih City only 23 trees species were used. Such a small number of species of trees and shrubs is incorrect. Therefore, in the future, it is necessary to increase the biological diversity of trees and shrubs for forest park stand.

As known, steppe ecological conditions (dry climate and lack of moisture), as well as technogenesis environmental conditions (anthropogenic activities, pollution of air and soil) negatively effect on the trees life. However, the dendrometric parameters of forest park stands (stem density, stem height, stem diameter, stand volume, stand basal area) in Background ecological areas were

similar to the natural forest (Lafleur et al., 2018; Kuuluvainen and Gauthier, 2018). This phenomenon can only be explained by the location of some parks.

The findings of our study clearly show that in Buffer and Impact ecological areas anthropogenic activities adversely affect to dendrometric parameters. Wherein, in Buffer areas the stem and values were by 10-20% more than in the Background areas. While in Impact areas these values were by 10-30 % lower than in Background areas. Therefore, it can be assumed that small anthropogenic activity and so-so habitat conditions stimulate the dendrometric parameters, and large anthropogenic activity and poor habitat conditions inhibit the dendrometric parameters.

The diversity and evenness have always been some of the most frequently used measures for forest stand and communities (Kaufman et al, 2017; Spake and Doncaster, 2017). The diversity and evenness index has been used as "indicators of the wellbeing of ecological systems". It should also be noted that protection of biodiversity and evenness of forests ecosystem is a strategic for sustainable forestry, understanding and gain an insight of natural forest stand. But the use of these indices is not widespread in modern ecological research.

The Shannon-Wiener diversity index has been the most widely used index in biology and ecology for levels diversity assessment (Kaufman et al, 2017; Santini et al, 2017). The values of this diversity index is usually found to fall from 0.0-5.0, usually between 1,5-3,5 and only rarely surpasses 4,5. The Shannon-Wiener diversity index increases as both the richness and the evenness of the community increase.

On forest park stands at Kryvyi Rih City the Shannon-Wiener diversity index values were ranged from 0,75 to 1,61. The species diversity showed significant variation between Background / Buffer and Background / Impact ecological areas (calculated by stem density). It should also be noted that the biodiversity levels of trees in the city forest park stands are low. The Pielou's evenness index expresses how evenly the individuals are distributed among the different species (Kaufman et al, 2017; Vacek, 2017). Usually this index is constrained between 0 and 1.0 with 1.0 representing a situation in which all species are equally abundant. On forest park stands at Kryvyi Rih City the Pielou's evenness index values were ranged from 0,53 to 0,86. The species evenness showed significant variation between Background / Buffer and Background / Impact ecological areas (calculated by stem density).

Simpson's diversity index calculates a diversity score for a community. It is based on both the number of different species in the community, and the number of individuals present for each of those species. The value of this index will always fall between 0 and 1, where 1 represents complete diversity and 0 represents complete uniformity (Kim, 2016; Santini et al, 2017; Spake and Doncaster, 2017). On forest park stands at Kryvyi Rih City the Simpson's diversity index (raging from 0,24 to 0,60) indicates to moderate trees species diversity.

Margalef's diversity index is a little popular in the current biological and ecological research. But this index measures species richness and it is highly sensitive to sample size and it is a very simple index to apply (Gamito, 2010; Konijnendijk, 2018). On forest park stands at Kryvyi Rih City the Margalef's diversity index values were ranged from 0,87 to 7,77. The species evenness showed significant variation between Background / Impact ecological areas (calculated by all stem density, stand volume and stand basal area).

In generally, compound indices, including variations of all diversity and evenness index, are assumed to intelligibly integrate species richness and evenness into forest park stands. The results of our research (floristic composition, dendrometric parameters, diversity and evenness indexes) differ significantly with the data that were obtained in boreal forest (Darabi et al, 2014; Rapanoela et al, 2016), but they are comparable with the data obtained in the forest park stands (Miller et al, 2015; Ostoić et al, 2018). Our results also show that differences in floristic composition, dendrometric parameters, diversity and evenness indexes are greater between urban land uses than ecological conditions. This fact, as wel as other results support our hypothesis that current state of the urban forest park stands determined by the combined influence of ecological (soil fertility, soil moisture) and environmental factors (air pollution).

CONCLUSIONS

The floristic compositions of Kryvyi Rih City urban forest park stands (Central Ukraine) are poor. There are only 24 species that belong to 14 families and 12 genera. The absolute and relative dendrometric parameters of the woody plants comparable to forest stand at the boreal zone. Ecological and environmental conditions statistically significant affect these characteristics. At low impact levels, there are stimulations of dendrometric parameters. At high impact levels, there are inhibitions of dendrometric parameters. The Kryvyi Rih City urban forest park stands characterized nasty of diversity and evenness indexes. This indicates the ecological instability of these woody plant communities. Current state of the urban forest park stands determined by the combined influence of ecological (soil fertility, soil moisture) and environmental factors (air pollution).

REFERENCES

Anguluri R., Narayana P. (2017): Role of green space in urban planning: outlook towards smart cities. Urban Forestry and Urban Greening, 25: 58-65. DOI: 10.1016/j.ufug.2017.04.007

Bielyk Y., Savosko V., Lykholat Y., Heilmeier H., Grygoryuk I. (2020): Macronutrients and heavy metals contents in the leaves of trees from the devastated lands at Kryvyi Rih District (Central Ukraine). E3S Web of Conferences, 166: 01011. https://doi.org/10.1051/e3sconf/202016601011

Booth T. H. (2018): Species distribution modelling tools and databases to assist managing forests under climate change. Forest Ecology and Management, 430: 196-203. DOI: 10.1016/j.foreco.2018.08.019

Carvalho F. P. (2017): Mining industry and sustainable development: time for change. Food and Energy Security, 6 (2): 61–77. DOI: 10.1002/fes3.109

Chivulescu S., Leca, S., Silaghi D., Cristea V. (2018): Structural biodiversity and dead wood in virgin forests from Eastern Carpathians. Agriculture and Forestry, 64 (1): 177-188. DOI: 10.17707/AgricultForest.64.1.20

Dan Z., Haifeng Z., Xingyuan H., Zhibin R., Chang Z., Xingyang Y., Zhixia M., Peijiang W. (2016): Effects of forest type and urbanization on species composition and diversity of urban forest in Changchun, Northeast China. Urban Ecosystems, 19 (1): 455-473. DOI: 10.1007/s11252-015-0473-5

Darabi S. M., Kooch Y., Hosseini S. M. (2014): Dynamic of Plant Composition and Regeneration following Windthrow in a Temperate Beech Forest. International Scholarly Research Notices, 2014: 421457. DOI: 10.1155/2014/421457

Ebrahimigajoti T., Nosrati H., Razbanhaghighi A., Khanbabai M. (2013). Studying biodiversity of plant associations in Sutan-Chay Basin in Arasbaran, northwest of Iran. Agriculture and Forestry, 59 (1): 85-98.

Fedorovskiy V. D., Terlyga N. S., Yukhimenko Yu. S., Danilchuk O. V., Danilchuk N. M., Lapteva O. V. (2013): Specific composition and vital state of arboreal-shrub vegetation of parks and public gardens of Kryvyi Rih. Plant Introduction, 3: 73-79. (in Ukrainian)

Fedorovskiy V. D., Mazur A. E. (2007): Woody plants of Kryvorozhsky botanical garden. Prospect, Ukraine. [in Russian]

Gamito S. (2010): Caution is needed when applying Margalef diversity index. Ecological Indicators, 10: 550-551. DOI: 10.1016/j.ecolind.2009.07.006

IPNI (2020): The International Plant Names Index, http://www.ipni.org.

Janhäll S. (2015): Review on urban vegetation and particle air pollution – deposition and dispersion. Atmospheric Environment, 105: 130-137. DOI: 10.1016/j.atmosenv.2015.01.052

Kaufman S., Hauck M. Leuschner Ch. (2017): Comparing the plant diversity of paired beech primeval and production forests: Management reduces cryptogam, but not vascular plant species richness. Forest Ecology and Management, 400 (15): 58-67. DOI: 10.1016/j.foreco.2017.05.043

Kim G. (2016): Assessing urban forest structure, ecosystem services, and economic benefits on Vacant Land. Sustainability, 8: 679. DOI: 10.3390/su8070679

Kolbe S. E., Miller A. I., Cameron G. N. Culley T. M. (2016): Effects of natural and anthropogenic environmental influences on tree community composition and structure in forests along an urban-wildland gradient in southwestern Ohio. Urban Ecosystems, 19 (2): 915-938. DOI: 10.1007/s11252-016-0531-7

Konijnendijk C. C. (2018): A Forest of diversity. In: The Forest and the City. Future City, 9. 185-194. Springer, Cham. DOI: 10.1007/978-3-319-75076-7_13

Kuuluvainen T., Gauthier S. (2018): Young and old forest in the boreal: critical stages of ecosystem dynamics and management under global change. Forest Ecosystems, 5: 26. DOI: 10.1186/s40663-018-0142-2

Lafleur B., Fenton N. J., Simard M., Leduc A., Paré D., Valeria O., Bergeron Y. (2018): Ecosystem management in paludified boreal forests: enhancing wood production, biodiversity, and carbon sequestration at the landscape level. Forest Ecosystems, 5: 27. DOI: 10.1186/s40663-018-0145-z

Liquete C., Cid N., Lanzanova D., Grizzetti B., Reynaud A. (2016): Perspectives on the link between ecosystem services and biodiversity: the assessment of the nursery function. Ecological Indicators, 63: 249-257. DOI: 10.1016/j.ecolind.2015.11.058

Lykholat Y., Khromyk N., Ivanko I., Kovalenko I., Shupranova L., harytonov M. (2016a): Metabolic responses of steppe forest trees to altirude associated local environmental changes. Agriculture and Forestry, 62 (2): 163-171

Lykholat Y., Alekseeva A., Khromykh N., Ivanko I., Kharytonov M., Kovalenko I. (2016b): Assessment and prediction of viability and metabolic activity of Tilia platyphyllos in arid steppe climate of Ukraine. Agriculture and Forestry, 62 (3): 65-71.

Lykholat Y. V., Khromykh N. O., Lykholat T. Y., Didur O. O., Lykholat O. A., Legostaeva T. V., Kabar A. M., Sklyar T. V., Savosko V. M., Kovalenko I. M., Davydov V. R., Bielyk Yu. V., Volyanik K. O., Onopa A. V., Dudkina K. A., Grygoryuk I. P. (2019a): Industrial characteristics and consumer properties of Chaenomeles Lindl. hruits. Ukrainian Journal of Ecology, 9 (3): 132-137.

Lykholat Y. V., Khromykh N. O., Lykholat T. Y., Bobrova O. M., Didur O. O., Lykholat O. A., Sudak V. M., Legostaeva T. V., Gtytzaj Z. V., Kabar A. M., Savosko V. M., Kovalenko I. M., Davydov V. R., Suvorova K. M., Dudkina K. A., Kolinko O. M., Grygoryuk I. P. (2019b): Introduction success of less common species from the genus Berberis L. Ukrainian Journal of Ecology, 9 (4): 634-640. DOI: 10.15421/2019_801

Margalef R. (1958): Temporal succession and spatial heterogeneity in phytoplankton. Perspectives in Marine biology, Buzzati-Traverso (ed.). University of California Press, Berkeley, 323-347.

Martins B., Pereira A. N. (2018): Index for evaluation of public parks and gardens proximity based on the mobility network: A case study of Braga, Braganza and Viana do Castelo (Portugal) and Lugo and Pontevedra (Spain). Urban Forestry and Urban Greening, 34: 134-140. DOI: 10.1016/j.ufug.2018.06.014

McDonald J. H. (2014): Handbook of biolological statistics. Sparky house publishing, USA.

Miller R. W., Hauer R. J., Werner L. P. (2015): Urban forestry: Planning and managing urban greenspaces. Waveland Press, USA.

Nowak D. J., Hirabayashi S., Doyle M., McGovern M., Pasher J. (2018): Air pollution removal by urban forests in Canada and its effect on air quality and human health. Urban Forestry and Urban Greening, 29: 40-48. DOI: 10.1016/j.ufug.2017.10.019

Olander L. P., Johnston R. J., Tallis H., Kagan J., Maguire L. A., Polasky S., Urban D., Boyd J., Wainger L., Palmer M. (2018): Benefit relevant indicators: ecosystem services measures that link ecological and social outcomes. Ecological Indicators, 85: 1262-1272. DOI: 10.1016/j.ecolind.2017.12.001

Ostoić S. K., Fabio S. S., Borelli A. V. (2018): Urban forest research in the Mediterranean: a systematic review. Urban Forestry and Urban Greening, 31: 185-196. DOI: 10.1016/j.ufug.2018.03.005

Pielou E. C. (1966): The measurement of diversity in different types of biological collections. Journal of Theoretical Biology, 13: 131-144.

Pimentel-Gomes F., Garcia C. H. (2002). Statistics applied to agronomic experiments and forest: Exposure with examples and guidelines for application use. FEALQ, Piracicaba, 309.

Polupan M. I., Solovej V. B., Velichko V. A. (2005): Classification of soils at Ukraine. Agrarian Science, Kyiv. (in Ukrainian)

Rapanoela R., Raulier F., Gauthier S. (2016): Regional instability in the abundance of open stands in the boreal forest of Eastern Canada. Forests, 7: 103. DOI:10.3390/f7050103

Rode J., Wittmer H., Emerton L., Schröter-Schlaack C. (2016): 'Ecosystem service opportunities': a practice-oriented framework for identifying economic instruments to enhance biodiversity and human livelihoods. Journal for Nature Conservation, 33: 35-47. DOI: 10.1016/j.jnc.2016.07.001

Santini S. L., Belmaker J., Costello M. J., Pereira H. M., Rossberg A. G., Schipper A. M., Ceausu S., Dornelas M., Hilbers J. P., Hortal J., Huijbregts M. A. J., Navarro L. M., Schiffers K. H., Visconti P., Rondinini C. (2017): Assessing the suitability of diversity metrics to detect biodiversity change. Biological Conservation, 213: 341-350. DOI: 10.1016/j.biocon.2016.08.024

Savosko V. M., Tovstolyak N. V. (2017): Ecological conditions of garden and park territories of former iron mines (Kryvyi Rih Basin, Ukraine). Ukrainian Journal of Ecology, 7 (4): 12–17. [in Ukrainian]

Savosko V., Lykholat Y., Domshyna K., Lykholat, T. (2018): Ecological and geological determination of trees and shrubs' dispersal on the devastated lands at Kryvorizhya. Journal of Geology, Geography and Geoecology, 27 (1): 116-130. DOI: 10.15421/111837 [in Ukrainian]

Savosko V. M., Lykholat Y. V., Bielyk Yu. V., Lykholat T. Y. (2019a): Ecological and geological determination of the initial pedogenesis on devastated lands in the Kryvyi Rih Iron Mining & Metallurgical District (Ukraine). Journal of Geology, Geography and Geoecology, 28 (4): 738-746. DOI: https://doi.org/10.15421/111969

Savosko V. M., Tovstolyak N. V., Lykholat Yu. V., Grygoryuk I. P. (2019b): Variability of botanical and ecological indicators of woodlands in gardens & parks cultivated plant communities at Kryvyi Rih District. Biological Systems: Theory and Innovation, 10 (3): 13-26. [in Ukrainian]

Savosko, V., Podolyak, A., Komarova, I., Karpenko, A (2020): Modern environmental technologies of healthy soils contaminated by heavy metals and radionuclides. E3S Web of Conferences, 166: 01007. DOI: https://doi.org/10.1051/e3sconf/202016601007

Shannon C. E., Wiener W. (1949): The mathematical theory of communication. University of Illinois Press: USA.

Simpson E. H. (1949): Measurement of diversity. Nature, 163: 688.

Soil Survey Staff (2014): Keys to Soil Taxonomy, 12th Edition. USDA-Natural Resources Conservation Service, Washington DC.

Solomou A., Skoufogianni E. (2016): Alpha and Beta plant diversity in multispecies agroecosystems of central Greece. Agriculture and Forestry, 62 (4): 19-25. DOI: 10.17707/AgricultForest.62.4.03

Song X. P., Tan P. Y., Edwards P., Richards D. (2018): The economic benefits and costs of trees in urban forest stewardship: a systematic review. Urban Forestry and Urban Greening, 29: 162-170. DOI: 10.1016/j.ufug.2017.11.017

Spake R., Doncaster P. (2017): Use of meta-analysis in forest biodiversity research: key challenges and considerations. Forest Ecology and Management, 400 (15): 429-437. DOI: 10.1016/j.foreco.2017.05.059

Tarasov V. V. (2012): Flora of Dnipropetrovsk and Zaporozhye regions. Lyra, Ukraine. [in Ukrainian]

Terlyga N. S., Danylchuk O. V., Yukhimenko Yu. S., Fedorovskiy V. D., Danylchuk N. M. (2015): Cultivated dendroflora of parks and parks of Kryvyi Rih: historical aspects of formation and modern state. Bulletin of Kharkiv National Agrarian University: a series of biology, 2 (35): 93-101. (in Ukrainian)

Urošević S., Vuković M., Pejčić B., Štrbac N. (2018): Mining-metallurgical sources of pollution in eastern Serbia and environmental consciousness. Revista internacional de contaminación ambiental, 34 (1): 103-115. DOI: 10.20937/RICA.2018.34.01.09

Vacek Z. (2017): Structure and dynamics of spruce-beech-fir forests in Nature Reserves of the Orlické hory Mts. in relation to ungulate game. Central European Forestry Journal, 63: 23–34. DOI: 10.1515/forj-2017-0006

Verma P., Raghubanshi A. S. (2018): Urban sustainability indicators: challenges and opportunities. Ecological Indicators, 93: 282-291. DOI: 10.1016/j.ecolind.2018.05.007

Voigt A., Kabisch N., Wurster D., Dagmar H., Breuste J. (2014): Structural diversity: a multi-dimensional approach to assess recreational services in urban parks. AMBIO, 43: 480-491. DOI: 10.1007/s13280-014-0508-9

West P. W. (2009): Tree and Forest Measurement. Springer-Verlag, Germany.

World reference base for soil resources (2014): International soil classification system for naming soils and creating legends for soil maps. Food and Agriculture Organization of the United Nations, Rome.

Rajičić, V., Terzić, D., Perišić, V., Dugalić, M., Madić, M., Dugalić, G., Ljubičić, N. (2020): Impact of long-term fertilization on yield in wheat grown on soil type vertisol. Agriculture and Forestry, 66 (3): 127-138.

DOI: 10.17707/AgricultForest.66.3.11

Vera RAJIČIĆ, Dragan TERZIĆ, Vesna PERIŠIĆ, Marijana DUGALIĆ, Goran DUGALIĆ [2], Milomirka MADIĆ [3], Nataša LJUBIČIĆ [1]

IMPACT OF LONG-TERM FERTILIZATION ON YIELD IN WHEAT GROWN ON SOIL TYPE VERTISOL

SUMMARY

Experiments were carried out at stationary in Kragujevac, in Serbia, during the three growing seasons. The primary aim of the research was perceiving of the influence of long-term usage of the same amounts and rates of nitrogen, phosphorus and potassium on the yield and grain quality of winter wheat variety „Kruna". Uptake NPK uptake by wheat ranged from 120 kg ha^{-1} nitrogen, from 60 to 100 kg ha^{-1} phosphorus and 60 kg ha^{-1} potassium, respectively, depending on treatments type. The grain yield of the wheat was significantly lower in control (treatment without fertilizer). The three-year grain yield of winter wheat was the highest in the NP_1K and NP_2K (4.367 and 4.531 t ha^{-1}) treatments. In terms of investigated traits, particularly grain yield and test weight fertilizer expressed more efficiency in the 2014/2015. Variance analysis showed statistically very significant differences for grain yield, 1.000-grain weight and test weight between the vegetation seasons and very significant differences for grain yield and 1000-grain weight between the variants of fertilization. Variance analysis showed very significant differences for 1000-grain weight between the interaction of the vegetation seasons and variants of fertilization.

It could be concluded that the grain yield in all treatments in the 2014/2015 growing season was significantly greater than in the other examined years, mostly as the result of highly favorable weather conditions at major stages of plant development wheat.

Keywords: fertilization, mineral nutrition, yield, wheat, correlation.

INTRODUCTION

In the last few decades, large experimental material about mineral nutrition and fertilizing of wheat was gathered. However, taking into account the dominant influence of the mineral nutrition in the synthesis of primary production of the

[1]Vera Rajičić, (corresponding author: verarajicic@yahoo.com), Dragan Terzić, Vesna Perišić, Marijana Dugalić, University of Niš, Faculty of Agriculture, Kosančićeva 4, Kruševac, SERBIA.
[2]Nataša Ljubičić, BioSense Institute, Zorana Đinđića 1, Novi Sad, SERBIA.
[3]Goran Dugalić, Milomirka Madić, University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, SERBIA.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:19/05/2020 *Accepted:26/06/2020*

organic matter and yield forming, this problem, intensively researched until now, will stay in the focal point of researchers and practitioners as long as plants cultivated (Knežević et al., 2015). A constant advance in the selection and creation of new varieties represents the strong impetus to research of different problems of the mineral nutrition of wheat (Perišić et al., 2016; Khan et al., 2017; Djuric et al., 2018; Jordanovska et al., 2018; Terzić et al., 2018a; Rajičić et al., 2019). With the appearance of the new wheat variety (essentially distinguished in a number of useful traits, a higher yield potential on the first place), it turned out their demands are more pronounced according to mineral nutrition (Knežević et al., 2007; Jelić et al., 2016; Khan and Mohammad, 2016; Đekić et al., 2017a; Tmušić et al., 2018).

Winter wheat use relatively large amounts of mineral elements during vegetation and have high demands according to soil fertility (Jelić et al., 2014; Đekić et al., 2017b). From the numerous macroelements from the soil, wheat use nitrogen the most, something less potassium, even less phosphorus and the least sulfur, magnesium, and calcium (Milivojević et al., 2018). Amounts of nutritional elements from the soil used by wheat during vegetation mostly depend on the grain yield level and the vegetative mass. In Serbia, the most often amounts of nitrogen needed to be applied for high yield are ranged between 80-120 kg ha-1 depending on agrochemical properties of the soil (Malešević et al., 2010; Popović et al., 2011; Đekić et al., 2014; Jelic et al., 2015; Terzic et al., 2018b; Rajičić et al., 2019).

From the numerous macroelements, nitrogen, phosphorus, and potassium are the most important for wheat growth and development and in certain quantities and rates must be incorporated in almost all soil types, regardless it represents direct nutrient usage or usage in preceding cultivated species (Popović et al., 2011). Between the elements of mineral nutrition, the nitrogen has the greatest role in yield increases of cultivated species (Knežević et al., 2007; Đekić et al., 2014; Terzic et al., 2018b; Madić et al., 2019), especially in wheat (Jelic et al., 2015; Khan and Mohammad, 2016; Đekić et al., 2017a; Rajičić et al., 2020). Nitrogen is showing the greatest effect through the joint usage with phosphorus and potassium (Popović et al., 2011; Đekić et al., 2014; Jelić et al., 2014; Khan et al., 2017; Terzic et al., 2018b; Knežević et al., 2019; Rajičić et al., 2019).

The primary aim of the research was perceiving of the influence of long-term usage of the same amounts and rates of nitrogen, phosphorus and potassium on the yield and grain quality of winter wheat variety „Kruna“, during the three growing seasons (2012/13, 2013/14 and 2014/15) on location Kragujevac in Republic of Serbia.

MATERIAL AND METHODS

Experimental design

Effects of mineral nutrition efficiency of wheat have been studied at the stationary field trial of the Small Grains Research Centre at Kragujevac location, (44° 22′ N, 20° 56′ E, 173-220 m a. s. l.) of Šumadija district in Republic of

Serbia, for three years period from 2013 to 2015. The winter wheat cultivar used in the experiment was Kruna, the dominant cultivar in the production region of Šumadija in Republic of Serbia. Fertilization was regular and followed a long-time scheme. Wheat sowing was done on two separated stationary fields (A and B) with corn rotation system.

Because of appearance of new demanded cultivars at permanent changes in soil fertility level and environmental conditions, still exist need to researches mineral nutrition of wheat, as well as determine optimal rates and balanced nutrition ratios in concrete agro ecological conditions. The rates of nitrogen application were 120 kg ha^{-1} N and they were applied either individually or in combination with two phosphorus rates and a potassium fertilizer. A non-fertilized variant served as a control. Eight variants of mineral nutrition N (120 kg ha^{-1} N), P_1 (60 kg ha^{-1} P_2O_5), P_2 (100 kg ha^{-1} P_2O_5), NP_1 (120 kg ha^{-1} N and 60 kg ha^{-1} P_2O_5), NP_2 (120 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5), NP_1K (120 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O), NP_2K (120 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O) and untreated control were tested in the experiment. The fertilizers applied were complex NPK fertilizers (15:15:15), superphosphate (17% P_2O_5) and CAN (calcium ammonium nitrate) as a nitrogen fertilizer containing 27% N. Total amounts of phosphorus and potassium fertilizers and half the nitrogen rate are regularly applied during pre-sowing cultivation of soil.

Sowing in all three analyzed years was carried out in the second half of October, at spacing between rows with of 12 cm, with a seed density of 500 germinating grains per m^2. The trial was designed in a randomized block with five replications. The crops were harvested at full maturity using (dates of harvesting 17.07.2012, 15.07.2013 and 04.07.2014). Three parameters, namely grain yield (t ha^{-1}), 1000-grain weight (g) and test weight (kg hl^{-1}) were analyzed. Grain yield was harvested and reported at 14% moisture. The 1000-grain weight was determined using an automatic seed counter. Test weight is the weight of a measured volume of grain expressed in kilograms per hectoliter.

Soil analysis

The trial was set up on a vertisol soil in a process of degradation, with heavy texture and very coarse and unstable structure (humus 2.2%, nitrogen 0.13%, phosphorus 2.0 mg 100 g^{-1} soil, potassium 20.0 mg K2O 100 g^{-1} soil and pH in H_2O 5.19).

Statistical analysis

The results were analyzed by the method of analyzing the variance of a single-factorial trial (ANOVA) using the statistics module Analyst Program SAS/STAT (SAS Institute, 2000). The significance of differences in mean values of the treatments was tested by the LSD test. Relative dependence was defined through correlation analysis (Pearson's correlation coefficient), and the coefficients that were obtained were tested at the 5% and 1% levels of significance.

Meteorological conditions

This study was conducted over a three-year period in the Šumadija region, Central Serbia, on a Vertisol soil, at Kragujevac location (Table 1).

Table 1. Precipitation sum and average monthly temperature in Kragujevac, Serbia

Months	X	XI	XII	I	II	III	IV	V	VI	VII	Aver.
Mean monthly air temperature (°C)											
2012/13	13.5	9.5	1.7	2.9	4.0	6.5	13.4	18.2	19.9	21.9	11.15
2013/14	13.5	9.2	2.4	5.0	6.9	9.0	12.2	15.3	19.7	21.8	11.50
2014/15	12.6	9.1	3.5	3.0	3.2	6.7	19.8	17.4	19.9	24.4	11.96
Average	12.5	6.9	1.9	0.5	2.4	7.1	11.6	16.9	20.0	22.0	10.18
The amount of precipitation (mm)											
2012/13	56.2	17.7	16.4	65.8	84.4	102.9	41.2	70.8	85.4	60.6	601.4
2013/14	41.7	61.2	6.4	21.2	9.0	67.1	129	227	45.8	138.6	747.0
2014/15	50.4	18.9	98.7	44.9	46.2	98.8	35.8	93.6	113	25.4	625.7
Average	45.4	48.9	56.6	58.2	46.6	32.4	51.9	57.6	70.4	46.6	514.6

Data in Table 1 for the investigated period clearly indicate that meteorological conditions recorded high variability during research. The average air temperature was higher by 0.97°C, 1.32°C and 1.78°C in 2012/13, 2013/14 and 2014/15 than the average of many years. The sum of rainfall precipitation was higher by 86.8 mm, 232.4 mm and 111.1 mm in 2012/13, 2013/14 and 2014/15 than the average of many years and with a very uneven distribution of precipitation per months. During the April and May in 2013/14 it more of rainfall what for 77.1 mm and 169.4 mm compared with the perennial average. In addition to the necessary reserve for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil (Dodig et al., 2008; Hristov et al., 2011; Iftikhar et al., 2012; Jelic et al., 2015; Terzić et al., 2018a; Grčak et al., 2019; Biberdzic et al., 2020).

RESULTS AND DISCUSSION

Grain yield, 1000-grain weight and test weight

Table 2 presents average values for grain yield, 1000-grain weight and test weight across years and treatments during the study. The highest three-year wheat grain yield of 3.787 t ha^{-1} was recorded in vegetation season 2014/15 and it was significantly higher than the yield in 2013/14 (2.382 t ha^{-1}). The three-year grain yield significantly varied across treatments, from 1.156 t ha^{-1} in control to 4.531 t ha^{-1} in treatment NP_2K with 120 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O.

The average three-year 1000-grain weight significantly varied across years from 37.19 g in 2014/15 to 40.77 g in 2012/13. The 1000-grain weight of winter wheat significantly varied across treatments, from 34.87 g in control to 41.01 g in treatment in treatment NP_2K with 120 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O.

The highest three-year wheat test weight in year 2014/15 (76.34 kg hl^{-1}) was significantly higher compared to 2012/13 and 2013/14 (71.80 and 72.25 kg

hl^{-1}). The highest test weight had the wheat application in treatment NP_1K in a quantity of 120 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O (74.54 kg hl^{-1}).

Table 2. The mean values for traits analyzed in Kragujevac, Serbia

Years	Grain yield (t ha^{-1})		1000-grain weight (g)		Test weight (kg hl^{-1})	
	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S
2012/13	3.233^{B*}	1.445	40.77^{A}	2.505	71.80^{C}	2.236
2013/14	2.382^{C}	0.934	39.30^{AB}	2.278	72.25^{B}	2.510
2014/15	3.787^{A}	1.569	37.19^{B}	3.502	76.34^{A}	2.346
Treatments						
C	1.156^{c}	0.498	34.87^{c}	3.620	72.79^{a}	3.308
N	2.837^{b}	0.690	39.97^{ab}	2.135	73.58^{a}	3.322
P_1	2.226^{b}	0.619	38.34^{b}	2.917	72.37^{a}	3.059
P_2	2.247^{b}	1.242	38.61^{b}	2.140	73.69^{a}	3.330
NP_1	3.803^{a}	1.156	40.59^{a}	2.140	73.36^{a}	2.518
NP_2	3.904^{a}	0.933	40.97^{a}	1.853	73.73^{a}	3.254
NP_1K	4.367^{a}	1.053	38.33^{b}	2.702	74.54^{a}	3.516
NP_2K	4.531^{a}	1.075	41.01^{a}	2.646	73.65^{a}	2.764

*Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test

Table 3 presents average values for grain yield, 1000-grain weight and test weight across years and treatments during the study. The yield during the 2012/13 significantly varied across treatments and the highest yield of 4.385 t ha^{-1} and 4.779 t ha^{-1} of was recorded in the NP_1K and NP_2K treatments. During the 2013/14 year, the yield significantly varied across treatments, and the highest average grain yield was recorded in the NP2K treatment (3.456 t ha^{-1}). During the 2014/15 grain yield significantly varied across treatments and the highest yield was in the NP_1K and NP_2K treatments (5.493 and 5.357 t ha^{-1}).

The 1000-grain weight of winter wheat significantly varied across all years and treatments as presented in Table 3. During the 2012/13 and 2013/14 1000-grain weight significantly varied across treatments and the highest 1000-grain weight was in the NP_2K treatment (43.36 g and 41.62 g). During the 2014/15 1000-grain weight significantly varied across treatments from 31.16 g in control to 40.84 g in NP_2. The contribution of fertilization in the 1000-grain weight was 15% more at control (treatment without fertilizer) compared to N treatment (under low or high nitrogen content) in grain yield variation (Rajičić et al., 2019).

The test weight in 2012/13 year varied across treatments, from 70.25 kg hl^{-1} in P_2 to 73.41 kg hl^{-1} in NP_2K treatments (Table 3). The higher values for test weight in 2013/14 year were found in the P_2 treatment (73.97 kg hl^{-1}). During the third year of examination (2014/15), significantly higher values for test weight were found in the NP_1K treatment (78.48 kg hl^{-1}).

The present results confirm the opinion of many authors that the GY and TGW are genetically determined but are strongly modified by the weather

conditions and soil nutrient availability (Đekić et al., 2014; Jelić et al., 2014; Khan and Mohammad, 2016; Khan et al., 2017; Terzic et al., 2018b; Knežević et al., 2019; Rajičić et al., 2020).

Table 3. Mean values for traits analyzed of years and fertilization in Kragujevac, Serbia

2012/13	Grain yield (t ha^{-1})		1000-grain weight (g)		Test weight (kg hl^{-1})	
	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S
C	1.006^{d}	0.338	39.20^{b}	0.485	70.73^{a}	1.197
N	2.970^{bc}	0.683	42.14^{a}	0.297	72.13^{a}	2.305
P_1	2.381^{c}	0.590	40.16^{b}	1.335	70.77^{a}	3.012
P_2	2.501^{c}	2.131	39.92^{b}	1.616	70.25^{a}	2.289
NP_1	3.785^{ab}	0.261	42.28^{a}	0.973	72.69^{a}	1.931
NP_2	4.054^{ab}	0.692	42.52^{a}	0.968	72.37^{a}	1.988
NP_1K	4.385^{a}	0.482	36.62^{c}	3.268	72.05^{a}	2.638
NP_2K	4.779^{a}	0.615	43.36^{a}	0.713	73.41^{a}	1.565
2013/14						
C	0.806^{f}	0.186	34.24^{c}	0.532	72.45^{a}	2.698
N	2.338^{cd}	0.363	39.98^{b}	0.657	71.17^{a}	1.368
P_1	1.855^{de}	0.584	40.24^{ab}	1.276	71.25^{a}	2.209
P_2	1.656^{ef}	0.249	39.52^{b}	1.045	73.97^{a}	2.270
NP_1	2.738^{bc}	0.508	39.26^{b}	1.571	71.89^{a}	2.308
NP_2	2.986^{ab}	0.243	39.56^{b}	1.499	72.37^{a}	3.457
NP_1K	3.224^{ab}	0.524	39.98^{b}	1.083	73.09^{a}	1.972
NP_2K	3.456^{a}	0.538	41.62^{a}	0.350	71.80^{a}	3.647
2014/15						
C	1.657^{c*}	0.473	31.16^{e}	2.054	75.18^{ab}	4.122
N	3.203^{b}	0.754	37.80^{bc}	1.912	77.43^{ab}	1.738
P_1	2.442^{bc}	0.625	34.62^{d}	0.646	75.09^{b}	2.184
P_2	2.584^{bc}	0.379	36.40^{cd}	1.744	76.85^{ab}	1.020
NP_1	4.886^{a}	1.211	40.24^{ab}	2.585	75.49^{ab}	2.051
NP_2	4.671^{a}	0.831	40.84^{a}	1.824	76.46^{ab}	2.685
NP_1K	5.493^{a}	0.392	38.38^{abc}	2.575	78.48^{a}	1.625
NP_2K	5.357^{a}	1.001	38.06^{abc}	2.373	75.73^{ab}	1.110

*Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test

Analysis of variance the analyzed traits

The analysis variance of yield, 1000-grain weight and test weight of wheat depending on the level of nitrogen, phosphorus and potassium fertilization on the yield and grain quality of wheat at Kragujevac in Serbia during three growing seasons, are shown in table 4. Data in Table 4 for the investigated period (2013-2015) clearly indicate that highly significant effect of year was found on grain yield (F=11.069**), 1000 grain-weight (F=16.445**) and test weight (F=44.664**). Furthermore, grain yield (F=24.371**) and 1000-grain weight (F=9.528**) was highly significant among the fertilization. The 1000-grain weight was highly significant regarding the interaction years and different treatments. The present results confirm the opinion of many authors that the grain

yield and 1000-grain weight are genetically determined but are strongly modified by the weather conditions and soil nutrient availability (Đekić et al. 2014, 2017a; Jelic et al., 2014, 2015; Khan et al., 2017; Milivojević et al., 2018; Popovic et. al., 2017; Terzić et al., 2018a; Tmušić et al., 2018; Rajičić et. al., 2019). Application of mineral fertilizers has a significant impact on 1000-grain weight which is significantly higher in more intensively fertilized variants as observed by Đekić et al. (2014), Terzić et al. (2018a) and Rajičić et al. (2020).

Table 4. The analysis of variance for the tested parameters in Kragujevac, Serbia

Effect	df	Mean sqr Effect	Mean sqr Error	F	p-level
The analysis of variance for grain yield					
Year, (Y)	2. 117	20.014	1.808	11.069**	0.000
Fertilization, (F)	7. 112	21.695	0.890	24.371**	0.000
Year x Fertilization, (YxF)	14. 96	0.629	0.530	1.188	0.297
The analysis of variance for 1000-grain weight					
Year, (Y)	2. 117	130.056	7.908	16.445**	0.000
Fertilization, (F)	7. 112	63.204	6.634	9.528**	0.000
Year x Fertilization, (YxF)	14. 96	17.105	2.535	6.747**	0.008
The analysis of variance for test weight					
Year, (Y)	2. 117	250.198	5.602	44.664**	0.000
Fertilization, (F)	7. 112	6.417	9.919	0.647	0.716
Year x Fertilization, (YxF)	14. 96	5.631	5.538	1.017	0.444

nsnon significant; *significant at 0.05; **significant at 0.01;

Table 5. Correlations between for traits analyzed by vegetation seasons

Correlations between the traits analyzed in 2012/13			
Traits	Grain yield (t ha^{-1})	1000-grain weight (g)	Test weight (kg hl^{-1})
Grain yield (t ha^{-1})	1.00	0.219ns	0.374*
1000-grain weight (g)		1.00	0.170ns
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in 2013/14			
Grain yield (t ha^{-1})	1.00	0.683**	-0.022ns
1000-grain weight (g)		1.00	-0.143ns
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in 2014/15			
Grain yield (t ha^{-1})	1.00	0.733**	0.094ns
1000-grain weight (g)		1.00	0.565ns
Test weight (kg hl^{-1})			1.00

nsnon significant; *significant at 0.05; **significant at 0.01;

Correlations between the analyzed traits

Table 5 presents the correlation coefficients between the years and examined traits. Positive and significant correlation coefficients, in 2012/13, were found between grain yield and test weight (r=0.374*). Highly significant and positive correlation coefficients, in 2013/14, as presented in Table 5, were found

between grain yield and 1000-grain weight (r=0.683**). Highly significant and positive correlation coefficients, in 2014/15, were found between grain yield and 1000-grain weight (r=0.733**), Table 5. The correlative dependence of the grain yield in the vegetation seasons was positive and highly significant with 1000 grain weight as established by Iftikhar et al. (2012), Đekić et al. (2014) and Terzić et al. (2018a). Grain yield depends directly on the number of grains per spike and the 1000 grain weight (Hristov, 2011; Iftikhar et al., 2012; Khan and Mohammad, 2016; Đekić et al., 2017a; Khan et al., 2017; Djuric et al., 2018; Terzić et al., 2018a).

Table 6. Correlation coefficients for the traits analyzed across treatments

Correlations between the traits analyzed in the unfertilized control			
	Grain yield (t ha^{-1})	1000-grain weight (g)	Test weight (kg hl^{-1})
Grain yield (t ha^{-1})	1.00	-0.299^{ns}	0.383^{ns}
1000-grain weight (g)		1.00	-0.440^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the N			
Grain yield (t ha^{-1})	1.00	-0.365^{ns}	0.344^{ns}
1000-grain weight (g)		1.00	-0.596*
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the P_1			
Grain yield (t ha^{-1})	1.00	-0.216^{ns}	0.186^{ns}
1000-grain weight (g)		1.00	-0.707**
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the P_2			
Grain yield (t ha^{-1})	1.00	-0.067^{ns}	0.188^{ns}
1000-grain weight (g)		1.00	-0.628*
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_1			
Grain yield (t ha^{-1})	1.00	0.471^{ns}	0.286^{ns}
1000-grain weight (g)		1.00	-0.044^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_2			
Grain yield (t ha^{-1})	1.00	0.536*	0.193^{ns}
1000-grain weight (g)		1.00	-0.145^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_1K			
Grain yield (t ha^{-1})	1.00	-0.162^{ns}	0.603*
1000-grain weight (g)		1.00	0.021^{ns}
Test weight (kg hl^{-1})			1.00
Correlations between the traits analyzed in the NP_2K			
Grain yield (t ha^{-1})	1.00	-0.133^{ns}	0.468^{ns}
1000-grain weight (g)		1.00	-0.316^{ns}
Test weight (kg hl^{-1})			1.00

^{ns}non significant; *significant at 0.05; **significant at 0.01;

Table 6 presents the correlation coefficients between the treatments and examined traits. The correlative dependence of the grain yield in the treatment NP_2 in was positive and medium significant correlation with the 1000-grain weight ($r=0.536^{*}$). Positive correlations were observed between grain yield and test weight in all treatments. Negative and medium significant correlation coefficients were found between 1000-grain weight and test weight in treatments N ($r=-0.596^{*}$) and P_2 ($r=-0.628^{*}$) and negative but strong highly significant correlation were in the treatment P_1 ($r=-0.707^{**}$).

The present results confirm the statement of many authors that the traits analyzed and their correlations are genetically determined but are strongly modified by the nutrient status of the environment and weather conditions (Đekić et al., 2014; Khan and Mohammad 2016; Terzic et al., 2018b; Lakić et al., 2018; Nazarenko et al., 2019; Rajičić et al., 2019).

CONCLUSIONS

Because of appearance of new demanded cultivars at permanent changes in soil fertility level and environmental conditions, still exist need to researches mineral nutrition of wheat, as well as determine optimal rates and balanced nutrition ratios in concrete agro ecological conditions. Effects of mineral nutrition efficiency of wheat have been studied at the stationary field trial of the Small Grains Research Centre in Kragujevac (Serbia) from 2012/2013 to 2014/2015. Nitrogen had a most significant impact on the yield of wheat. The average grain yield of all cultivars in the 2014/15 growing season was significantly greater than in the following years, mostly as the result of highly favourable weather conditions at major stages of plant development. The highest average yields were gained by NP1K (4.367 t ha-1) and NP2K (4.531 t ha-1) treatment in three-year period.

The analysis of variance showed very significant differences of vegetation seasons on the grain yield, 1000-grain weight and test weight. The analysis of variance showed statistically highly significant differences for grain yield and 1000-grain weight between the fertilization. Mineral nutrition in study significantly increased the yield of winter wheat.

ACKNOWLEDGEMENTS

This paper is part of the projects, Grant numbers: 451-3-68/2020-14/200189 and 200358, financed by the Ministry of Education, Science and Technology Development of Republic of Serbia.

REFERENCES

Biberdzic, M., Barac, S., Lalevic, D., Djikic, A., Prodanovic, D., Rajicic**,** V. (2020): Influence of soil tillage system on soil compactionand winter wheat yield. Chilean Journal of Agricultural Research, 80 (1): 80-89.

Dodig D., Zorić M., Knežević D., King S., Šurlan-Momirović G. (2008): Genotype x environment interaction for wheat yield in different drought stress conditions and agronomic traits suitable for selection. *Aust. J. Agr. Res.*, 59: 536-545.

Đekić V., Milovanović M., Popović V., Milivojević J., Staletić M., Jelić M., Perišić, V. (2014): Effects of fertilization on yield and grain quality in winter triticale. Rom. Agric. Res., 31: 175-183.

Đekić V., Jelić M., Milivojević J., Popović V., Branković S., Staletić M., Terzić D. (2017a): Winter wheat yield and yield components depending on the level of nitrogen, phosphorus and potassium fertilization. Proceedings, VIII International Scientific Agriculture Symposium "Agrosym 2017", 05-08 October, Jahorina, 650-657.

Đekić V., Milivojević J., Jelić M., Popović V., Branković S., Biberdžić M., Terzić D. (2017b): Effects of fertilization on production traits of Takovčanka cultivar of winter wheat. Proceedings, 2nd International and 14th National Congress of Soil Science Society of Serbia "Solutions and Projections for Sustainable Soil Management". NSoil 2017, 25-28th September 2017, Novi Sad, Serbia, 49-54.

Djuric N., Prodanovic S., Brankovic G., Djekic V., Cvijanovic G., Zilic S., Dragicevic V., Zecevic V., Dozet G. (2018): Correlation-Regression Analysis of Morphological-Production Traits of Wheat Varieties. Rom. Biotech. Lett. 23 (2): 13457-13465.

Grčak M., Grčak D., Grčak D., Aksić M., Gudžić S., Nikolić K., Rajičić V. (2019): Phosphorus, potassium and nitrogen abundance in soil of Doljevac municipalit. Proceedings, X International Scientific Agriculture Symposium "Agrosym 2019", Jahorina, October 03-06, 2019, 1169-1173.

Hristov N., Mladenov N., Kondić-Špika A. (2011): Effect of environmental and genetic factors on the correlation and stability of grain yield components in wheat. Genetika, 43 (1): 141-152.

Iftikhar R., Khaliq I., Ijaz M., Rahman A.M. (2012): Association analysis of grain yield and its components in spring wheat (*Triticum aestivum* L.). Amer. Euras. J. Agric. Envir. Sci., 12 (3): 289-392.

Jelić M., Milivojević J., Đekić V., Paunović A., Tmušić N. (2014): Impact of liming and fertilization on grain yield and utilization of nitrogen and phosphorus in wheat plant grown on soil type pseudogley. Proceedings of research papers PKB Agroekonomik, Belgarde, 20 (1-4): 49-56.

Jelic M., Milivojevic J., Nikolic O., Djekic V., Stamenkovic S. (2015): Effect of long-term fertilization and soil amendments on yield, grain quality and nutrition optimization in winter wheat on an acidic pseudogley. Rom. Agric. Res., 32: 165-174.

Jelić M., Milivojević J., Paunović A., Madić M., Đekić V. (2016): Adaptation of winter wheat genotypes to low ph and high mobile al content in the soil. Proceedings, VII International Scientific Sympozium "Agrosym Jahorina 2016", AGROSYM, 06-09. October, Jahorina, 762-767.

Jordanovska S., Jovović Z., Dolijanović Ž., Dragičević V., Branković G., Đekić V. (2018): Nutritional properties of Macedonian landraces of small grain cereals as a source of new genetic variability. Genetika, 50 (3): 863-883. doi: 10.2298/GENSR1803863J

Knezevic D., Paunovic A., Madic M., Djukic N. (2007): Genetic analysis of nitrogen accumulation in four wheat cultivars and their hybrids. Cereal Res. Commun., 35 (2): 633-336.

Knežević D., Radosavac A., Zelenika M. (2015): Variability of grain weight per spike of wheat grown in different ecological conditions. Acta Agriculturae Serbica, 20 (39): 85-95.

Knežević D., Paunović A., Kondić D., Radosavac A., Laze A., Kovačević V., Mićanović D. (2019): Variability in seed germination of barley cultivars (*Hordeum vulgare* L.) grown under different nitrogen application rates. Acta Agriculturae Serbica, 24 (47): 61-69. doi: 10.5937/AASer1947061K

Khan F.U. and F. Mohammad (2016): Application of stress selection indices for assessment of nitrogen tolerance in wheat (*Triticum aestivum* L.). The J. Anim. Plant Sci., 26 (1): 201-210.

Khan F.U., Mohammad F., Raziuddin. Z., Shah M., Ahmad M., Shah Z. (2017): Genotypic differences and genotype × nitrogen interactions for yield traits in bread wheat. The J. Anim. Plant Sci., 27 (4): 1264-1268.

Lakić Ž., Glamočlija Đ., Kondić D., Popović V., Pavlović S. (2018): Krmne biljke i žita u funkciji zaštite zemljišta od degradacije. Monografija. Poljoprivredni fakultet Banja Luka. ISBN 978-99938-93-47, p. 1 - 405.

Madić, M., Knežević, D., Đurović, D., Paunović, A., Stević, V., Tomić, D., Đekić V. (2019): Assessment of the correlation between grain yield and its components in spring barley on an acidic soil. Acta agriculturae Serbica, 24 (47): 41-49. doi: 10.5937/AASer1947041M

Malešević M., Glamočlija Đ., Pržulj N., Popović V., Stanković S., Živanović T., Tapanarova A. (2010): Production characteristics of different malting barley genotypes in intensive nitrogen fertilization. Genetika, Belgrade, 42(2): 323-330.

Milivojević J., Bošković-Rakočević Lj., Đekić V., Luković K., Simić Z. (2018): Cultivar-specific accumulation of iron, manganese, zinc and copper in winter wheat grain (*Triticum aestivum* L.). J. Centr. Eur. Agric., 19 (2): 423-436. doi: 10.5513/JCEA01/19.2.2051

Nazarenko, M., Mykolenko, S., Chernysky, V. (2019): Modern Ukrainian winter wheat varieties grain productivity and quality at ecological exam. Agriculture and Forestry, 65 (1): 127-136. DOI:10.17707/AgricultForest.65.1.13

Perišić V., Perišić V., Živanović T., Milovanović M., Đekić V., Staletić M., Luković K. (2016): Comparative stability analysis of yield components of wheat genotypes. Proceedings, XX International Eco-Conference® 2016, 9th Eco-Conference® on Safe Food, 28-30. September 2016, Novi Sad, Serbia, 63-72.

Popović, V., M. Tatić, V. Spalević, V. Rajičić, V. Filipović, LJ. Šarčević-Todosijević and P. Stevanović (2017): Effect of nitrogen fertilization on soybean plant height in arid year. Proceedings, 2nd International and 14th National Congress of Soil Science Society of Serbia "Solutions and Projections for Sustainable Soil Management". 25-28th September 2017, Novi Sad, Serbia, 65-73.

Rajičić V., Milivojević J., Popović V., Branković S., Đurić N., Perišić V., Terzić D. (2019): Winter wheat yield and quality depending on the level of nitrogen, phosphorus and potassium fertilization. Agriculture and Forestry, 65 (2): 79-88. DOI: 10.17707/AgricultForest.65.2.06

Rajičić, V., Popović, V., Perišić, V., Biberdžić, M., Jovović, Z., Gudžić, N., Mihailović, V., Čolić, V., Đurić, N., Terzić, D. (2020): Impact of Nitrogen and Phosphorus on Grain Yield in Winter Triticale Grown on Degraded Vertisol. *Agronomy*, 2020, 10 (6): 757.

SAS/STAT (2000): User's Guide, Version 9.1.3. SAS Institute Inc.

Terzić D., Đekić V., Milivojević J., Branković S., Perišić V., Perišić V., Đokić D. (2018a): Yield components and yield of winter wheat in different years of research. Biologica Nyssana, 9 (2): 119-131. doi: 10.5281/zenodo.2538604

Terzic D., Đekić V., Jevtic S., Popovic V., Jevtic A., Mijajlovic J., Jevtic A. (2018b): Effect of longterm fertilization on grain yield and yield components in winter triticale. The J. Anim Plant Sci. 28(3): 830-836.

Tmušić N., Knežević J., Đekić V., Nikolić K. (2018): The influence of weather conditions and fertilizing method on plant height at different cultivars of winter wheat. Proceedings, IX International Scientific Agriculture Symposium "Agrosym 2018", Jahorina, October 04-06, 2018; 385-389.

Giannoulis, K., Platis, I., Gougoulias, N., Wogiatzi, E. (2020): Influence of Trichoderma harzianum and Glomus mycorrhiza on biomass and essential oil yield of organic Ocimum basilicum cultivation. Agriculture and Forestry, 66 (3): 139-154

DOI: 10.17707/AgricultForest.66.3.12

Kyriakos GIANNOULIS[1], Ilias PLATIS[2], Nikolaos GOUGOULIAS[2], Eleni WOGIATZI[2]

INFLUENCE OF *Trichoderma harzianum* AND *Glomus mycorrhiza* ON BIOMASS AND ESSENTIAL OIL YIELD OF ORGANIC *Ocimum basilicum* CULTIVATION

SUMMARY

The growing interest in dealing with the cultivation of aromatic – medicinal plants requires the study of factors related to the production process, which includes the development of plants in field, as well as their processing and the receipt of essential oils. The aim of this study was to investigate the effect of two organic formulations, *Trichoderma harzianum* (T22), and Glomus mycorrhiza (G), in the cultivation of seedlings of *Ocimum basilicum* variety "Genovese" fresh and dry weight, as well as essential oil content and its quality characteristics. For the purposes of the study a field experiment was conducted in 2018 and 2019, using a factorial experimental design, with two factors: a) control, b) *Trichoderma harzianum* (T22) and c) *Mycorizas Glomus* (G) under four replicates. There was found statistically significantly difference between the two harvests occurred in each year on fresh and dry weight while no significant difference was recorded through the tested treatments. Furthermore, there was recorded a decrease in the essential oil yield between the cultivating years (approximately 14%), and also between annual cuts.

Finally, the most remarkable finding was that although the different treatments did not show significant differences in the quantitative characteristics of sweet basil in the case of quality characteristics with *mycorrhiza* treatments essential oil having 40 identified ingredients instead of 21 that identified in control. Therefore, the use of these organic formulations is suggested to be used only in case of essential oil production where a predetermined quality of essential oil will be known.

Keywords: aromatic-medicinal plants, *Ocimum basilicum*, organic plant production, yield, essential oil

[1]Giannoulis Kyriakos, (corresponding author: kyriakos.giannoulis@gmail.com), University of Thessaly, Dept of Agriculture, Crop Production and Rural Environment, Laboratory of Agronomy and Applied Crop Physiology, Fytokoy Str., 38446 Volos, GREECE.

[2]Platis Ilias, Gougoulias Nikolaos, Wogiatzi Eleni, University of Thessaly, Department of Agrotechnology, 41110, Larissa, GREECE.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Received:03/07/2020 *Accepted:05/08/2020*

INTRODUCTION

Ocimum basilicum is a herbaceous plant of Lamiaceae family (Grayer et al., 1996) with annual and perennial species. It is one of the most popular aromatic plants and is widely used in cooking (Chalchat and Ozcan, 2008), pharmaceutical (Ahmed et al., 2014), confectionery and perfumery (Nguyen et al., 2010). Basil (*Ocimum basilicum* L.) in the science of medicine is called Herba Basilici and it is the most economically important species (Bravo et al., 2008).

It is considered to be native to the tropical and subtropical zones of Africa and Asia. It was firstly spread to India and other regions of Asia (Klimánková et al., 2008) and later on to Greece and the Mediterranean basin (Marotti et al. 1996; Sanda et al. 1998; Martins et al. 1999). It appeared in North America around 1620 through the first European settlers.

Ocimum genus includes more than 50 species. *Ocimum basilicum* is an economically and industrially important plant. It is a versatile plant with many different morphological characteristics, such as the size, the color and the texture of the leaves, the color of the inflorescence, but also the chemical composition of the essential oil. Basil has a smooth or slightly fluffy shoot, multi-branched with a height ranging up to 1 m (Blank et al., 2004). Its growth in width is almost the same as in height.

It is cultivated in areas with temperate climates, mild and short winters and cool summers. Basil requires high growth temperatures, with an excellent of 25°C. At temperatures below 7°C, especially in the early growing stages, the damage is irreversible. It is cultivated in rich and light soils, with a pH that can range from 4.5-8.2 (with an excellent value of 6.4), warmly sunny, irrigated and well drained. It has high water requirements, especially on hot summer days, but it needs attention to excessive watering.

Transplanting takes place in April-May at distances of 30-40 x 40-50 cm, by hand or with automatic transplanting machines in properly prepared soil. Before transplanting the plants are pruned at a height of 15 cm to encourage the growth of lateral branches. The broadleaf basil does not stop growing even at flowering stage.

The essential oil is stored in glandular trichomes of leaves (Sangwan et al., 2001) and it is used in fungicides or insecticides and in various pharmaceutical and industrial products (Grayer et al., 1996). The global production of basil essential oil varies from 50 to 100 tones (Lubbe and Verpoorte, 2011), while it is reported that Indian basil essential oil yield varies from 132.0 to 162.5 kg ha^{-1} (Singh et al., 2010). Basil essential oil contains antifungal anti-bacterial properties and it is used as aromatic agents in food, perfumery and pharmaceutical industries (Varban et al., 2010; Wannissorn et al., 2005). The qualitative and quantitative improvement of essential oil production represents an area of high commercial interest (Copetta et al., 2006).

Nowadays, there is a high interest for organic products. In literature is reported investigation on different bio-fertilizers and/or symbiosis with fungi (Augé, 2001; Xavier and Boyetchko, 2002; Lee et al., 2013; Bharti et al., 2016) in

case to increase the quantitative and qualitative characteristics of the yield, especially in the case of aromatic and pharmaceutical plants. The main goal of the studies that examine mycorrhizal colonization is to improve plants production (Sun et al., 2017; Gheisari et al., 2017). There are also few studies where is tested the symbiosis of arbuscular mycorrhiza and basil under water stress (Copetta et al., 2006; Hazzoumi et al., 2017).

Nowadays there is an increasing interest in cultivating basil in Greece and in Mediterranean basin in general, under organic production system. There has been created intense concern for the investigation of factors that can increase basil quantitative and qualitative characteristics. The aim of this study is to investigate the effect of two biological preparations, *Trichoderma harzianum* (T 22), and *Glomus mycorrhiza* (G) on *Ocimum basilicum* variety Genovese yield and essential oil.

MATERIALS AND METHODS

A field experiment was established on 2018 and 2019 at the Experimental Farm of the General Department of the University of Thessaly (Larissa plain, 39°62′69″ N, 22°38′14″ E). Basil seedlings of the most cultivated variety Genovese were transplanted on 12/05/2019 and 13/05/2019 in a row distance of 40 cm and plant distance 20 cm. The initial height of the established plants (seedlings) was 5 cm. There was used a complete randomized experimental designs with three factors and four replicates. The tested factors were: i) control, ii) *Trichoderma harzianum* (T22), and iii) *Glomus mycorrhiza* (G). The applications of *Trichoderma harzianum* and *Glomus mycorrhizae* were performed during the transplanting stage applying 5ml/plant and 5gr/plant of T22 and G, respectively. There was installed a drip irrigation system with self-adjusting drippers of two-liter per hour in a distance of 60 cm. Irrigation was carried out every seven days and lasting four hours at a time. There was not used any fertilization while the weed management took pat manually in both years.

Table 1. Physicochemical soil properties.

Characteristics	Depth 0-30 cm
pH	7.75
Organic matter (%)	1.17
Electrical conductivity (mS/cm)	0.08
CEC (cmol/kg)	16.7
P-Olsen (ppm)	9.6
N-inorganic (ppm)	112
K-exchangeable (ppm)	214
Na-exchangeable (ppm)	71

Before seedling establishment on 2018 a soil sampling up to a depth of 30 cm for further analysis carried out and the soil physicochemical characteristics are presented in Table 1.

Sampling carried out with two cuts on 06/07/20018 and on 05/09/2018 for the first experimental year and on 04/07/2019 and on 04/09/2019 for the second. Sampling took place at flowering stage and the plants were cut 4 cm above ground. In order to avoid any border effect, samplings were done from the 1 m^2 of the inner rows of the plot of each replication. The plot size was 20 m2 (4 m width x 5 m length). The fresh weight was measured at the field and then the samples were transported to the laboratory for air drying and further analysis.

Twelve and a half grammars of basil dry leaves were subjected to 250 ml of water in a Clevenger-type distillation apparatus in case to determine the essential oil content. The hydro distillation lasted 105 minutes. The procedure was repeated three times for each sample and the essential oil content was estimated on the basis of DW plant material (ml 100 g^{-1}). A further analysis of the distilled essential oil analysis was done using a GC-MS on a fused silica DB-5 column and a Gas chromatograph interfaced with a mass spectrometer. The relative content of each compound was calculated as a percentage (%) of the total chromatographic area and the results are expressed as the mean percentage (%) of three replicates (Sarrou et al., 2017; Tsivelika et al., 2018).

The climatic data were recorded by an automatic meteorological station that was located near the experimental field. Finally all data were analyzed using GenStat (7th Edition) package and the $LSD_{.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Weather conditions

The study area is characterized by a typical Mediterranean climate with cold humid winters and hot-dry summers.

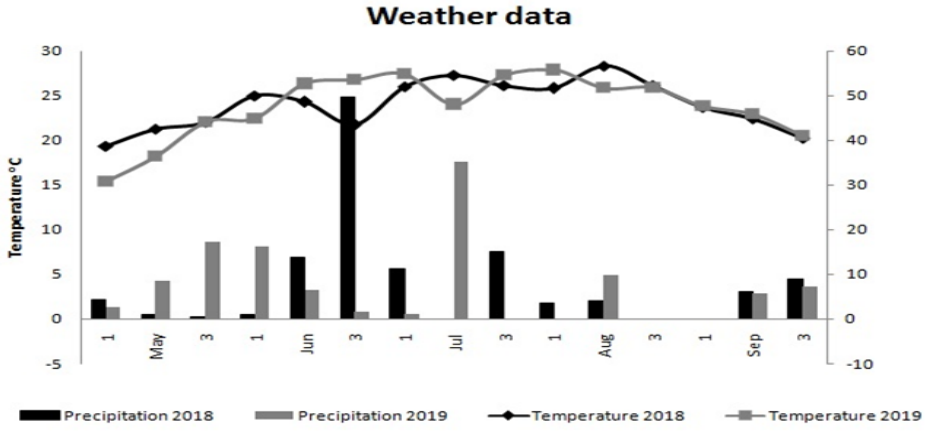


Figure 1. Average temperature and precipitation occurred in the study site during the growing periods of *Ocimum basilicum* (2018 and 2019).

In particular, the average air temperature ranged during the transplanting period (May) was about 20.9 and 18.5°C on 2018 and 2019, respectively, while during summer months (June-August) the average air temperature was about 26 for both years (Fig. 1). Precipitation during May was only 5.7 mm on 2018 and 28.5 mm on 2018, while during the summer months (June-August) was 98 mm and 70 mm on 2018 and 2019, respectively (Fig. 1).

Basil fresh and dry yield

The fact that the time intervals between the transplanting of the plants and the first cut, as well as between the first and second cut are the same and equal to two months in 2018 and 2019, and there was not observed extremely differences to the weather data, make the results comparable.

Table 2. Trichoderma and mycorrhiza application effect on basil fresh weight.

Treatments	Total Weight	Leaves Weight	Stems Weight
	t ha^{-1}		
T22	14.52	8.91	5.61
G	14.19	8.49	5.69
Control	14.13	8.67	5.46
$LSD_{0.05}$	ns	ns	ns
2018	16.71	10.27	6.44
2019	11.84	7.11	4.74
$LSD_{0.05}$	0.701	0.501	0.325
1st Cut	16.61	10.66	5.95
2nd Cut	11.95	6.72	5.23
$LSD_{0.05}$	1.114	0.755	0.441
T22 x 2018	16.67	10.34	6.33
T22 x 2019	12.38	7.48	4.90
G x 2018	16.57	10.04	6.53
G x 2019	11.80	6.95	4.85
Control x 2018	16.91	10.45	6.46
Control x 2019	11.35	6.89	4.46
$LSD_{0.05}$	ns	ns	ns

T22 x 1st Cut	16.42	10.55	5.87
T22 x 2nd Cut	12.63	7.27	5.36
G x 1st Cut	16.56	10.53	6.03
G x 2nd Cut	11.81	6.46	5.35
Control x 1st Cut	16.85	10.90	5.95
Control x 2nd Cut	11.41	6.44	4.97
$LSD_{0.05}$	ns	ns	ns
2018 x 1st Cut	20.94	13.49	7.45
2019 x 2nd Cut	12.49	7.06	5.43
2018 x 1st Cut	12.28	7.83	4.45
2019 x 2nd Cut	11.41	6.38	5.02
$LSD_{0.05}$	1.261	0.867	0.522
T22 x 2018 x 1st Cut	20.26	13.05	7.21
T22 x 2018 x 2nd Cut	13.09	7.63	5.45
T22 x 2019 x 1st Cut	12.58	8.06	4.52
T22 x 2019 x 2nd Cut	12.17	6.90	5.27
G x 2018 x 1st Cut	20.78	13.35	7.43
G x 2018 x 2nd Cut	12.35	6.72	5.63
G x 2019 x 1st Cut	12.34	7.71	4.64
G x 2019 x 2nd Cut	11.27	6.20	5.07
Control x 2018 x 1st Cut	21.77	14.07	7.70
Control x 2018 x 2nd Cut	12.04	6.82	5.22
Control x 2019 x 1st Cut	11.92	7.73	4.19
Control x 2019 x 2nd Cut	10.78	6.05	4.73
$LSD_{0.05}$	ns	ns	ns
CV (%)	12.9	14.3	13.0

The total fresh and/or dry weight did not statistical differ for the tested treatments in both cultivating years (Table 2 and Table 3). The only statistical significant differences that were found are between the cultivating years and the different cuts in the same year. There was observed a reduction in the fresh weight during the second experimentation year and between the first and second cut for both years, at rates of 30% and 28% respectively. These reductions were not statistically significant. The reduction in the fresh weight observed between the cuttings (1st and 2nd) during the second year (2019) was considered to be

lower. Finally, it was found that leaves fresh and dry weight was higher comparing to stems weight for both years (Table 2).

In many studies has been shown that by increasing the available nitrogen significantly increases the fresh weight of plants, up to an excellent one (Anwar et al., 2005a; Sifola and Barbieri, 2006; Giannoulis et al., 2020), which was not found in the current study although it could be expect that this would happen due to the use of Trichoderma and Mycorrhiza that contribute to a better nutrient uptake from the soil. Almost the same results were reported by Kandil et al., (2009) who found that the fresh weight of basil Genovese variety had no significant differences among fertilized plots with 25, 50, 75 and 100% from recommended doses of N-P-K.

As it has been reported the yield of 2018 was higher compared with the produced yield in 2019 reaching an average yield of 16.7 t ha^{-1} in total of which 10.3 t ha^{-i} were the fresh leaves (Table 2). The contained moisture was for all parts the same 84%.

Table 3. Trichoderma and mycorrhiza application effect on basil dry weight.

Treatments	Total Weight	Leaves Weight	Stems Weight
	t ha^{-1}		
T22	2.63	1.62	1.01
G	2.54	1.52	1.02
Control	2.80	1.72	1.08
$LSD_{0.05}$	ns	ns	ns
2018	2.68	1.65	1.04
2019	2.63	1.59	1.07
$LSD_{0.05}$	ns	ns	ns
1st Cut	3.16	2.03	1.13
2nd Cut	2.15	1.21	0.94
$LSD_{0.05}$	0.225	0.151	0.086
T22 x 2018	2.65	1.64	1.00
T22 x 2019	2.62	1.59	1.02
G x 2018	2.54	1.54	1.00
G x 2019	2.54	1.51	1.03
Control x 2018	2.86	1.76	1.10

Control x 2019	2.73	1.67	1.06
$LSD_{0.05}$	ns	ns	ns
T22 x 1st Cut	3.06	1.97	1.09
T22 x 2nd Cut	2.20	1.27	0.93
G x 1st Cut	3.08	1.95	1.13
G x 2nd Cut	2.00	1.09	0.90
Control x 1st Cut	3.37	216.0	1.18
Control x 2nd Cut	2.25	1.27	0.98
$LSD_{0.05}$	ns	ns	ns
2018 x 1st Cut	3.27	2.11	1.16
2019 x 2nd Cut	2.09	1.19	0.91
2018 x 1st Cut	3.05	1.95	1.10
2019 x 2nd Cut	2.21	1.24	0.97
$LSD_{0.05}$	ns	ns	ns
T22 x 2018 x 1st Cut	3.18	2.05	1.13
T22 x 2018 x 2nd Cut	2.11	1.23	0.88
T22 x 2019 x 1st Cut	2.95	1.89	1.06
T22 x 2019 x 2nd Cut	2.29	1.30	0.99
G x 2018 x 1st Cut	3.21	2.06	1.15
G x 2018 x 2nd Cut	1.87	1.02	0.85
G x 2019 x 1st Cut	2.95	1.84	1.11
G x 2019 x 2nd Cut	2.13	1.17	0.95
Control x 2018 x 1st Cut	3.43	2.22	1.21
Control x 2018 x 2nd Cut	2.30	1.31	0.99
Control x 2019 x 1st Cut	3.24	2.10	1.14
Control x 2019 x 2nd Cut	2.21	1.24	0.97
$LSD_{0.05}$	ns	ns	ns
CV (%)	14.0	15.4	13.7

The dry herbage yield reported in previous studies where different sweet basil genotypes were tested varied from 0.6 to 6.2 t ha^{-1} yield (Bowes and Zheljazkov, 2004; Anwar et al., 2005b; Zheljazkov et al., 2008b; Zheljazkov et

al., 2008a). The above variation reflects the different genetically, agronomical and environmental effects and is within the yield variations reported in this study.

Essential oil yield

The most important quality criterion of aromatic-medicinal plants is essential oil. The essential oil content was found to have a statistically significant higher content for the harvested yield in 2018 compared to the one measured in 2019 (0.47 and 0.43% respectively, Table 4) and also between the two cuts in each (0.8 and 0.1% in the 1st and the 2nd cut, respectively; Table 4). There was not found any statistically significant difference between the treatments or the interactions with the cuts and the growing year (Table 4).

Table 4. *Trichoderma* and *mycorrhiza* application effect on basil essential oil content and yield.

Treatments	Essential oil Content	Essential Oil Yield
	%	kg ha^{-1}
T22	0.44	8.34
G	0.49	8.28
Control	0.46	9.54
$LSD_{0.05}$	ns	ns
2018	0.47	9.36
2019	0.43	8.08
$LSD_{0.05}$	0.037	0.814
1st Cut	0.80	16.21
2nd Cut	0.10	1.24
$LSD_{0.05}$	0.030	0.900
T22 x 2018	0.47	9.07
T22 x 2019	0.41	7.61
G x 2018	0.47	9.01
G x 2019	0.43	7.55
Control x 2018	0.47	10.01
Control x 2019	0.45	9.08
$LSD_{0.05}$	ns	ns

T22 x 1st Cut	0.77	15.51
T22 x 2nd Cut	0.09	1.17
G x 1st Cut	0.79	15.33
G x 2nd Cut	0.11	1.23
Control x 1st Cut	0.82	17.78
Control x 2nd Cut	0.10	1.31
$LSD_{0.05}$	ns	ns
2018 x 1st Cut	0.83	17.41
2019 x 2nd Cut	0.11	1.32
2018 x 1st Cut	0.77	15.00
2019 x 2nd Cut	0.09	1.16
$LSD_{0.05}$	ns	1.15
T22 x 2018 x 1st Cut	0.82	16.78
T22 x 2018 x 2nd Cut	0.11	1.36
T22 x 2019 x 1st Cut	0.75	14.24
T22 x 2019 x 2nd Cut	0.08	0.97
G x 2018 x 1st Cut	0.82	16.84
G x 2018 x 2nd Cut	0.12	1.18
G x 2019 x 1st Cut	0.75	13.82
G x 2019 x 2nd Cut	0.11	1.28
Control x 2018 x 1st Cut	0.84	18.61
Control x 2018 x 2nd Cut	0.11	1.41
Control x 2019 x 1st Cut	0.81	16.94
Control x 2019 x 2nd Cut	0.10	1.21
$LSD_{0.05}$	ns	ns
CV (%)	11.1	17.0

The lowest (0.08% in 2019 during the second cut to the *Trichoderma*; Table 4) and the highest (0.84% in 2018 during the first cut to the control; Table 4) essential oil content was found in the case of *Trichoderma* and control, respectively. However, the most remarkable finding was that both *Trichoderma* and *mycorrhiza* had the same essential oil content in the first cut for both growing years.

Multiplying leaves dry yield per hectare with the essential oil content of each treatment, result to the essential oil yield per hectare. This yield is presented

in Table 4 and shows that the yield of 2018 is statistically significantly higher compared to the yield of 2019 (9.36 and 8.08 kg ha^{-1}, respectively). This difference is much higher comparing the two cuts independently growing year with the second cut producing thirteen times less essential oil yield per hectare (Table 4).

The increased essential oil content during the summer months has been observed in numerous studies of many aromatic plants of the *Lamiaceae* family (Putievsky et al. 1986; Kokkini et al. 1997). It seems that the content of essential oils shows seasonal diversity, with a decrease in autumn and higher amounts in summer. The role of temperature and UVB radiation is very important (Fahlen et al. 1997; Ioannidis et al. 2002), as well as the light duration and intensity, which has an increasing effect on essential oil production (Yamaura et al. 1989). Thus the reason that the second cut in the study area had extremely lower essential oil content.

The range of the essential content of the current study was within the usual content reported in other studies where it is reported that the essential oil content of different sweet basil genotypes varying from 0.07% to 1.92% in dry herbage (Pino et al., 1994; Wetzeil et al., 2002; Bowes and Zheljazkov, 2004.; Anwar et al., 2005b, Zheljazkov et al., 2008a). The above essential oil content corresponds to a basil oil yield ranging between 3.1 kg ha^{-1} and 58.8 kg ha^{-1}, a range that includes the essential oil yield of this study.

Essential oil composition

The essential oil analysis shown that in the produced essential oil were identified 40 ingredients (Table 5). The main ones recorded high concentrations (according to control treatments) are methyl chavicol (51.5%), cadinol (7.6%), geranial (7.3%), linalool (7.1%), a-guaiene (4.4%), c-cadiene (2.7%) and cis-Cadina 1(6) – 4 diene (2.5%).

The most remarkable finding was that although differential treatments did not show significant differences in the quantitative characteristics of sweet basil cultivation, in the case of quality characteristics this difference seems to be important. As it is presented in Table 5, there have been many differences between the treatments and the control in the contents of the individual ingredients. The ingredients that identified to the essential oil of the *mycorrhiza* treatments were 40 instead of 21 and 19 that identified in control and *trichoderma*, respectively.

The essential oil of the *mycorrhiza* treatments has more ingredients that even in small quantities make it richer. Furthermore, it must be emphasized that there was found also a high difference to the % content of the found compound (Table 5).

Previous studies have assessed the chemical composition of essential oils of *O. basilicum* presenting a high variability (Edris and Farrag, 2003; Rattanachaikunsopon and Phumkhachorn, 2010).

Table 5. Essential oil compounds found to the tested treatments (average values for both.

Ingredient (%)	Control	Trichoderma	Mycorrhiza
a-Pinene	0	0	0.014
Camphlene	0	0	0.004
Sabinene	0	0	0.035
b-Pinene	0	0	0.366
Mycerene	0	0	0.065
Lauroene	0.452	0.418	0.420
1,8 Cineole	0.765	0.712	0.701
Z-b Ocimene	0	0	0.006
E-b- Ocimene	0.616	0.583	0.534
C-Terpinene	0	0	0.015
cis-Terpinene hydrate	0	0	0.020
cis Linalool oxide	0	0	0.027
Linalool	7.083	7.209	6.696
1-octen-3-yl acetate	0	0	0.006
Menthone	0	0	0.063
Borneol/isomethone	0	0	0.296
Menthol	0	0	0.029
Terpinen-4-ol	0	0	0.058
a-Terpineol	0	0	0.065
Methyl chavicol	51.497	56.603	56.030
Geraniol	0	0	0.804
Geranial	7.280	2.888	2.089
Bornyl Acetate	0	0	0.013
Methyl Acetate	0.134	0	0.141
d-Elemene	0.016	0	0.010
a-Cubanene	0.096	0	0.035
a-Copaene	1.257	1.344	1.071
b-Elemene	0.123	0.147	0.149
a-Guaiene	4.358	4.750	4.066
a-Humuulene	3.076	2.677	2.365
cis-Cadina 1(6) - 4 diene	2.508	3.469	2.350
b-Acoradiene	0.735	0	0.522
c-Muurolene	0.759	0.742	0.630
b-Selinere	0.470	0.472	0.420
Aciphylene	2.445	2.352	2.058
c-Bulnesene	0.908	0.971	0.866
c-Cadiene	2.733	2.674	2.632
Spathulenol	0	1.054	1.149
1,10 di-epi Cubenol	0	0.986	0.903
Cadinol	7.612	7.423	6.535

According to the different genotype of *O. basilicum*, there were found different compounds content to linalool (Hussain et al., 2008; Pozzatti et al., 2008; Rao et al., 2011), eugenol (Nardoni et al., 2015), geraniol (Cardoso et al., 2016) and methyl chavicol (Opalchenova and Obreshkova, 2003).

Finally in previous studies was reported essential oil of *O. basilicium* with high amounts of methyl chavicol 45–86% (Bozin et al., 2006; Hossain et al., 2010; Shirazi et al., 2014; Císarová et al., 2016; Avetisyan et al., 2017), which is in line with the findings of the current study.

CONCLUSIONS

This study was conducted during 2018 and 2019 seasons, at the General Department of the University of Thessaly, to study the effect of two biological preparations, *Trichoderma harzianum* (T 22), and *Glomus mycorrhiza* (G) on *Ocimum basilicum* variety Genovese yield and essential oil. The results indicated that the above treatments did not affect the quantitative characteristics but only the qualitative ones. Moreover, sweet basil yield was different between the cuts with the first one producing the major yield. Therefore, an overall conclusion could be that sweet basil could be a promising annual aromatic-medicinal crop and different essential oil quality could be produced depending on the use of or not of mycorrhiza or trichoderma according to market requirements for its use.

ACKNOWLEDGMENTS

This work was supported by the General Department, University of Thessaly, Greece.

REFERENCES

Ahmed EA, Hassan EA, Tobgy KM, Ramadan EM. 2014. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. Ann. Agric. Sci. 59(2):273–280.

Anwar F, Ashraf M, Bhanger MI. 2005a. Interprovenance variation in the composition of *Moringa oleifera* oil seeds from Pakistan. J Am. Oil Chem. Soc. 82:45–51.

Anwar M, Patra DD, Chand S, Kumar A, Naqvi AA, Khanuja SPS. 2005b. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. Commun. Soil Sci. Plant Anal. 36:1737–1746.

Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42.

Avetisyan A, Markosian A, Petrosyan M, Sahakyan N, Babayan A, Aloyan S, Trchounian A, 2017. Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. BMC Complement. Altern. Med. 17:60.

Bharti N, Barnawal D, Wasnik K, Tewari SK, Kalra A. 2016. Co-inoculation of *Dietzia natronolimnaea* and *Glomus intraradices* with vermicompost positively influences *Ocimum basilicum* L. growth and resident microbial community structure in salt affected low fertility soils. Appl. Soil Ecol. 100:211–225.

Blank AF, Carvalho Filho JLS, Santos Neto AL, Alves PB, Arrigoni-Blank MF, Silva-Mann R, MendonćEa MC. 2004. Caracterizacão morfológica e agronômica de acessos de manjericão e alfavaca. Hortic. Bras. 22:113–116.

Bowes K, Zheljazkov V.D. 2004. *Ocimum sanctum* L. and *Ocimum basilicum* L. grown in Nova Scotia, Canada show potential as essential oil crops. J. Amer. Soc. Hort. Sci. 129:789–794.

Bozin B, Mimca-Dukic N, Simin N, Anackov G. 2006. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem. 54:1822–1828.

Bravo E, Amrani S, Aziz M, Harnafi H, Napolitano M. 2008. *Ocimum basilicum* ethanolic extract decreases cholesterol synthesis and lipid accumulatio in human macrophages. Fitoterapia 79:515–523.

Cardoso NNR, Alviano CS, Blank AF, Romanos MTV, Fonseca BB, Rozental S, Rodrigues IA, Alviano DS. 2016. Synergism effect of the essential oil from *Ocimum basilicum* var. Maria Bonita and its major components with fluconazole and its influence on ergosterol biosynthesis. Evid. Based. Complement. Alternat. Med. 2016:5647182.

Chalchat JC, Ozcan MM. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. Food Chem. 110:501–503.

Císarová M, Tančinová D, Medo J, Kačániová M. 2016. The in vitro effect of selected essential oils on the growth and mycotoxin production of Aspergillus species. J. Environ. Sci. Heal. Part B. 51:668–674.

Copetta A, Lingua G, Berta G. 2006. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. Mycorrhiza 16:485–494.

Edris AE, Farrag ES. 2003. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Nahrung–Food. 47:117–121.

Fahlen A, Welander M, Wennersten R. 1997. Effects of light-temperature regimes on plant growth and essential oil yield of selected aromatic plants. J. Sci. Food Agric. 73:111–119.

Gheisari S, Movahhedi M, Salehi A, Gholamhoseini M. 2017. Responses of field grown fennel (*Foeniculum vulgare* Mill.) to different mycorrhiza species under varying intensities of drought stress. J. Appl. Res. Med. Aromatic Plants, 5:16–25.

Giannoulis KD, Kamvoukou CA, Gougoulias N, Wogiatzi E. 2020. Irrigation and nitrogen application affect Greek oregano (*Origanum vulgare* ssp. *hirtum*) dry biomass, essential oil yield and composition. Ind. Crops Prod. 150:112392.

Grayer RG, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. 1996. Infraspecific taxonomy and essential oil chemotypes in basil, *Ocimum basilicum*. Phytochemistry 43:1033–1039.

Hazzoumi Z, Moustakime Y, Elharchli E, Joutei K. 2017. Effect of *arbuscular mycorrhizal* fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (*Ocimum gratissimum* L.). Chem. Biol. Technol. Agric. 2:10.

Hossain MA, Kabir MJ, Salehuddin SM, Rahman SMM, Das AK, Singha SK, Alam MK, Rahman A. 2010. Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh. Pharm. Biol. 48:504–511.

Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 108:986–995.

Ioannidis D, Bonner L, Johnson CB. 2002. UN-B is required for normal development of oil glands in *Ocimum basilicum* L. (sweet basil). Ann. Bot. 90:453-460.

Kandil MAM, Khatab ME, Ahmed SS, Schnug E. 2009. Herbal and essential oil yield of Genovese basil (*Ocimum basilicum* L.) grown with mineral and organic fertilizer sources in Egypt. Journal FöR Kulturpflanzen, 61(12):443–449.

Klimánková E, Holadová K, Hajšlová J, Cájka T, Poustka J, Koudela M. 2008. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chem. 107:464–472.

Kokkini S, Karousou R, Dardioti A, Krigas N, Lanaras T. 1997. Autumn essential oils of Greek oregano. Phytochemistry, 44:883-886.

Lee EH, Eo JK, Ka KH, Eom AH. 2013. Diversity of arbuscular mycorrhizal fungi and their roles in ecosystems. Mycobiology 41(3):121–125.

Lubbe A, Verpoorte R. 2011. Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind. Crops Prod. 34(10):785-801.

Marotti M, Piccaglia R, Giovanelli E. 1996. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J. Agr. Food Chem., 14:3926–3929.

Martins AP, Salgueiro LR, Vila R, Tomi F, Canigueral S, Casanova J, Proença da Cunha A, Adzet T. 1999. Composition of the essential oils of *Ocimum canum*, *O. gratissimum* and *O. minimum*. Planta Med. 65:187–189.

Nardoni S, Giovanelli S, Pistelli L, Mugnaini L, Profili G, Pisseri F, Mancianti F. 2015. In vitro activity of twenty commercially available, plant-derived essential oils against selected dermatophyte species. Nat. Prod. Commun. 10:1473–1478.

Nguyen PM, Kwee EM, Niemeyer ED. 2010. Potassium rate alters the antioxidant capacity and phenolic concentration of basil (*Ocimum basilicum* L.) leaves. Food Chem. 123:1235–1241.

Opalchenova G, Obreshkova D. 2003. Comparative studies on the activity of basil - an essential oil from *Ocimum basilicum* L. - against multidrug resistant clinical isolates of the genera Staphylococcus, Enterococcus and Pseudomonas by using different test methods. J. Microbiol. Methods 54:105–110.

Pino JA, Roncal E, Rosado A, Goire I. 1994. The essential oil of *Ocimum basilicum* L. from Cuba. J. Essent. Oil Res. 6:89–90.

Pozzatti P, Scheid LA, Spader TB, Atayde ML, Santurio JM, Alves SH. 2008. In vitro activity of essential oils extracted from plants used as spices against fluconazoleresistant and fluconazole-susceptible Candida spp. Can. J. Microbiol. 54:950–956.

Putievsky E, Ravid U, Duda N. 1986. The essential oil and yield components from various plant parts of *Salvia fruticosa*. J. Nat. Prod. 49(6):1015-1017.

Rao BRR, Kotharia SK, Rajput DK, Patel RP, Darokar MP. 2011. Chemical and biological diversity in fourteen selections of four *Ocimum* species. Nat. Prod. Commun. 6:1705–1710.

Rattanachaikunsopon P, Phumkhachorn P. 2010. Antimicrobial activity of basil (*Ocimum basilicum*) oil against salmonella enteritidis in vitro and in food. Biosci. Biotechnol. Biochem. 74:1200–1204.

Sanda K, Koba K, Nambo P, Gaset A. 1998. Chemical investigation of *Ocimum* species growing in Togo. Flavour Fragr. J., 13:226–232.

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. 2001. Regulation of essential oil production in plants. Plant Growth Regul. 34:3–21.

Sarrou E, Tsivelika N, Chatzopoulou P, Tsakalidis G, Menexes G, Mavromatis A. 2017. Conventional breeding of Greek oregano (*Origanum vulgare ssp. hirtum*) and development of improved cultivars for yield potential and essential oil quality. Euphytica. 213:104.

Shirazi MT, Gholami H, Kavoosi G, Rowshan V, Tafsiry A. 2014. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of Tagetes minuta and Ocimum basilicum essential oils. Food Sci. Nutr. 2:146–155.

Sifola MI, Barbieri G. 2006. Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci. Hort. 108:408–413.

Singh S, Singh M, Singh AK, Kalra A, Yadav A, Patra D.D. 2010. Enhancing productivity of Indian basil (*Ocimum basilicum* L.) through harvest management under rainfed conditions of subtropical north Indian plains. Ind. Crops Prod. 32:601–606.

Snoussi M, Dehmani A, Noumi E, Flamini G, Papetti A. 2016. Chemical composition and antibiofilm activity of *Petroselinum crispum* and *Ocimum basilicum* essential oils against Vibrio spp. strains. Microb. Pathog. 90:13–21.

Steel RGD, Torrie JH. 1982. Principles and procedures of statistics. A Biometrical Approach, 2nd ed. McGraw-Hill, Inc. pp. 633.

Sun X, Shi J, Ding G. 2017. Combined effects of *arbuscular mycorrhiza* and drought stress on plant growth and mortality of forage sorghum. Appl. Soil Ecol. 119:384–391.

Tsivelika N, Sarrou E, Gusheva K, Pankou C, Koutsos T, Chatzopoulou P, Mavromatis A. 2018. Phenotypic variation of wild Chamomile (*Matricaria chamomilla* L.) populations and their evaluation for medicinally important essential oil. Biochem. Syst. Ecol. 80:21-28.

Varban DL, Duda MM, Varban R. 2010. The study of the *Ocimum basilicum* L. specie cultivated in organic system. Pro Environ. 3:284–288.

Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S. 2005. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia 76:233–236.

Wetzeil SB, Kruger H, Hammer K, Bachmann K. 2002. Investigations on morphological and molecular variability of *Ocimum* L. species. J. Herbs, Spices Med. Plants 9:8183–8187.

Xavier LJC, Boyetchko SM. 2002. *Arbuscular mycorrhizal* fungi as biostimulants and bioprotectants of crops. Appl. Mycol. Biotechnol. 2:311–340.

Yamaura T, Tanaka S, Tabata M. 1989. Light dependent formation of glandular trichomes and monoterpenes in thyme seedlings. Phytochemistry, 28:741-744.

Zheljazkov VD, Callahan A, Cantrell CL. 2008a. Yield and Oil Composition of 38 Basil (*Ocimum basilicum* L.) Accessions Grown in Mississippi. J. Agric. Food Chem. 56:241–245

Zheljazkov VD, Cantrell CL, Evans WB, Ebelhar MW, Coker C. 2008b. Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. HortScience. 43:737–741.

Haitlinger, R., Šundić, M. (2020): Cooremania kietaensis sp. nov. (Astigmata: Canestriniidae) from Bougainville Island, Papua New Guinea. Agriculture and Forestry, 66 (3): 155-168

DOI: 10.17707/AgricultForest.66.3.13

Ryszard HAITLINGER [1]***, Miloje ŠUNDIĆ*** [2]

COOREMANIA KIETAENSIS SP. NOV. (ASTIGMATA: CANESTRINIIDAE) FROM BOUGAINVILLE ISLAND, PAPUA NEW GUINEA

SUMMARY

Cooremania kietaensis sp. nov. from Papua New Guinea is described and illustrated, based on males, females and protonymphs. All specimens were obtained from undetermined Lucanidae (Coleoptera). All canestriniids known from Papua New Guinea are listed.

Keywords: Acari, taxonomy, new species, Lucanidae, Coleoptera, Papua New Guinea canestriniids.

INTRODUCTION

Insects constitute the most diverse form of animal life in terrestrial ecosystems (Bolu, 2016). The family Lucanidae (Coleoptera) includes about 1200 species in the world fauna which occurring mainly in subtropical regions. The canestriniid mites were found only on 47 lucaniid species and some undetermined species, 17 canestriniid species found on Lucanidae [without determined to the genus for example mentioned by Okabe and Goka (2008)] belonging to 9 genera: *Amboniophela* Haitlinger, 1993, *Canestrinia* Berlese, 1881, *Cooremania* Nesbitt, 1976, *Haitlingeria* Kim, Lee, Choi, Sim and Kim, 2006, *Noemiphela* Haitlinger, 1991, *Rugoniphela* Haitlinger, 1991, *Sandrophela* Haitlinger, 1990, *Uriophela* Haitlinger, 1991 and *Vereoxia* Haitlinger, 1995 (Berlese, 1881, Nesbitt, 1976, Haitlinger, 1990a, 1991, 1993, 1995, Kim et al., 2006).

One species of *Coleopterophagus* (Kishida, 1924) found on Lucanidae not belongs to this genus. Canestriniid mites were found on Lucanidae only in Asia, Europe and Papua New Guinea (Berlese, 1881, Kishida, 1924, Nesbitt, 1976, Haitlinger, 1989a, b, 1990a, 1993, 1995, Kim et al., 2006, Okabe and Goka 2008). From Papua New Guinea were known only two species associated with Lucanidae: *Cooremania wauensis* Nesbitt, 1976 and *Amboinophela bernae* Haitlinger, 1993 (Nesbitt, 1976, Haitlinger, 1993). Moreover, in Papua New

[1]Ryszard Haitlinger, (corresponding author: ryszard.haitlinger@upwr.edu.pl), Institute of Biology, Invertebrate Systematics and Ecology, Wroclaw University of Environmental and Life Sciences, Kozuchowska 5B, 51-631 Wroclaw, POLAND.

[2]Miloje Šundić, (miloje.sundic@gmail.com) Department of Biology, Faculty of Sciences, University of Montenegro, Cetinjski put b. b. 80 000 Podgorica, MONTENEGRO.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Received:24/06/2020 *Accepted:02/08/2020*

Guinea were found 20 other species: *Albinorattia igori* Haitlinger, 1989 associated with Cetoniinae (Scarabaeidae), *Sandrophela kokodaica* Haitlinger, 1990 associated with Passandridae, *Athogavia arrybasi* Haitlinger, 1989, *Aurilossongia bolanica* Haitlinger, 1989, *A. guarana* Haitlinger, 1989, *A. norae* Haitlinger, 1989, *Mossongia bissaina* Haitlinger, 1989, *M. savina* Haitlinger, 1989 associated with Dynastinae (Scarabaeidae), *Apalotacarus cidaris* Summers & Schuster, 1981, *A. fusulus* Summers & Schuster, 1981, *A. petilus* Summers & Schuster, 1981, *A. rigescens* Summers & Schuster, 1981, *Arraphosoma ninax* Summers & Schuster, 1981, *Jullongia izae* Haitlinger, 1990, *Lidiophela pecki* (Nesbitt, 1976), *Passalophagus georlei* Nesbitt, 1976, Phaleratus fentoni Nesbitt, 1976, *Photia howdeni* Nesbitt, 1976 and *Sajanophela alfredae* Haitlinger, 1989 associated with Passalidae (Nesbitt, 1976, Summers & Schuster, 1981a, b, Haitlinger, 1989a, b, 1990a, b).

The genus *Cooremania* Nesbitt, 1976 is known only from one species found in Papua New Guinea and differing from all other genera by the presence of peg-like setae on ventral part of idiosoma and legs I-III (in all instars). In this paper we describe a new species *Cooremania kietaensis* from Bougainville Island, Papua New Guinea. It is second species of the genus *Cooremania.*

MATERIALS AND METHODS

Three males, two females and three protonymphs of the new species were obtained from the Lucanidae collection of the Museum of Natural History, Wrocław University. All specimens were preserved in 75% ethanol. Mite specimens were cleared in Nesbitt's solution and mounted in Berlese's medium. Figures were drawn using a Carl Zeiss Axioscope A1 compound microscope. Measurements were made using a NIKON Eclipse 50i compound microscope. All measurements are given in micrometers. The terminology and abbreviations follow Grandjean (1939), Griffiths et al., (1990), Norton (1998), Trach and Khaustov (2011) and Haitlinger and Šundić (2016).

RESULTS

Canestriniidae Berlese, 1884

Cooremania Nesbitt, 1976

Type species: *Cooremania wauensis* Nesbitt, 1976, by original designation.

Diagnosis. Propodosomal and sejugal structure absent. The presence of peg-like setae on the ventral surface of all stages. Part of setae on legs I-III peg-like. Males with well developed adanal suckers.

Cooremania kietaensis sp. nov.

(Figs. 1-20)

Diagnosis. Setae 2a, c_3, 3a, 4b, ad_2 and g peg-like. Setae p_1, p_2, p_3 and h_3 tubercle-like in males, peg-like in females, setae vF I, vF II and nG peg-like.

Description. Male (holotype) (n = 3) – Idiosoma with ornamentation on propodosoma and anterior part of hysterosoma; Propodosoma with setae vi, si

and long setae se. Hysterosoma with relatively short setae c_1, c_2, d_1, d_2, e_1, e_2 and f_2 (Fig. 1).

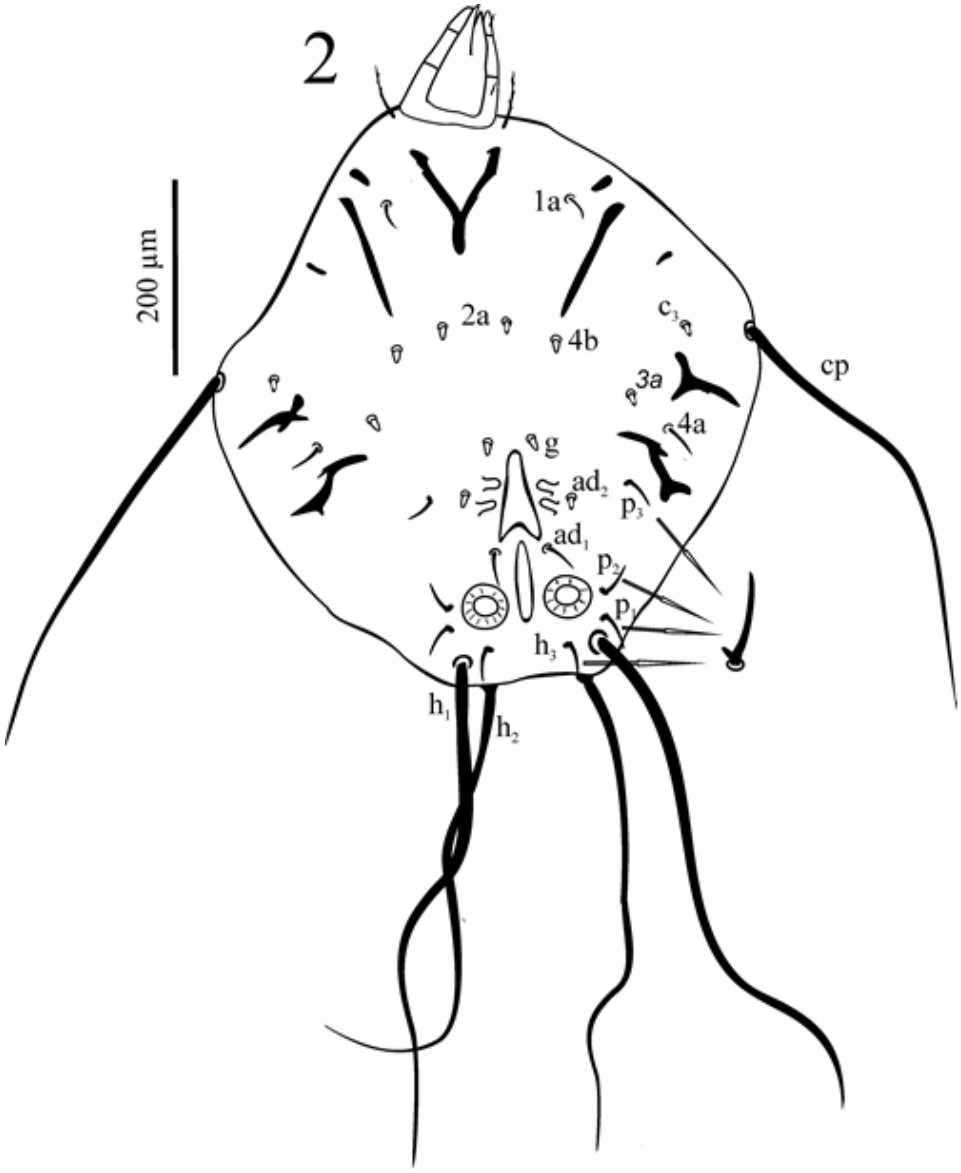
Among setae placed in middle of idiosoma distance $c_1 – c_1$ is the longest, distance $d_1 – d_1$ is longer than distance $e_1 – e_1$ and $e_2 – e_2$ (Table 1).

Table 1. Metric data of *Cooremania kietaensis* sp. nov.; H – holotype, P– paratype, PR – protonymphs.

Character	H ♂	P ♂	P ♂	P ♀	P ♀	PR	PR	PR
IL	526	480	595	730	677	407	481	430
IW	440	444	488	666	617	328	385	388
GL	108	91	100	110	106	90	87	97
GW	80	74	93	79	73	49	55	64
c_1	42	36	40	65	61	42	54	53
c_2	52	46	66	69	69	51	57	65
c_3	11	13	14	15	17	12	13	12
d_1	36	33	42	66	69	44	44	47
d_2	39	57	58	80	76	61	57	68
e_1	26	26	27	87	85	47	-	55
e_2	24	26	31	61	67	51	44	54
h_1	500	447	539	577	-	-	-	-
h_2	472	486	520	517	513	-	197	-
h_3	38	30	27	17	18	12	12	12
cp	425	409	435	490	523	-	-	243
se	390	-	425	498	-	310	-	391
si	54	63	77	75	73	56	52	52
vi	62	60	76	75	92	55	71	60
SW	34	36	44	-	-	-	-	-
SS	54	50	66	-	-	-	-	-
ANL	61	58	69	-	-	-	-	-
PL	69	60	72	-	-	-	-	-
p_1	37	36	32	16	12	15	12	12
p_2	32	30	27	18	13	12	12	12
p_3	23	22	23	13	14	18	18	16
1a	27	30	30	40	40	31	32	26
2a	15	15	15	16	16	11	13	15
3a	14	13	13	17	16	12	14	15
4a	29	29	36	13	18	19	-	-
4b	15	14	18	16	15	-	-	-
g	11	10	13	19	19	11	-	-
f_2	26	27	32	46	47			
Ta I	62	63	68	71	67	44	56	45
Ta II	56	56	64	66	67	49	50	50
Ta III	85	83	83	96	86	65	72	66
Ta IV	93	93	88	119	112	80	87	-
Leg I	221	195	222	227	210	137	157	160
Leg II	200	187	223	-	210	151	160	159
Leg III	235	239	250	264	258	198	192	200
Leg IV	261	229	262	304	282	179	192	-
Ta I ω_1	42	45	55	55	55	35	34	37
Ta I ω_2	11	11	12	15	12	12	10	18

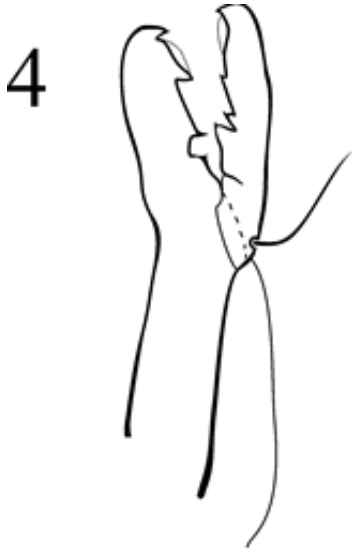
Ta I ω_3	78	66	83	71	82	50	59	60
Ta I d	95	81	100	92	100	79	88	71
Ta I e	40	45	46	46	40	33	35	32
Ta I wa	40	43	29	47	32	33	27	29
Ta I p	20	20	20	30	23	16	20	26
Ta I q	21	-	27	44	17	25	27	22
Ta I la	15	17	22	22	15	16	16	18
Ti I φ	151	152	-	172	171	120	-	126
Ge I σ	49	45	51	53	54	40	34	41
Ge I mG	26	25	33	31	26	18	24	20
Ge I cG	31	34	38	39	36	31	40	35
Fe I vF	7	9	8	9	11	11	9	11
Tr I vTr	35	43	37	22	24	32	33	31
Ta II ω	65	59	56	63	67	51	51	52
Ta II d	72	98	124	115	105	70	76	66
Ta II e	36	48	54	55	49	36	29	31
Ta II wa	29	44	46	39	35	*28*	23	28
Ta II ra	28	45	38	-	44	37	38	21
Ta II la	16	20	18	18	20	-	-	-
Ti II φ	73	70	83	79	82	55	61	69
Ge II σ	36	33	39	36	37	30	29	-
Ge II mG	33	25	37	37	32	18	20	22
Ge II cG	23	20	20	23	24	24	15	18
Fe II vF	10	14	10	12	10	8	10	12
Tr II vTr	35	27	20	30	36	29	-	-
Ta III d	48	67	57	127	117	84	73	-
Ta III e	45	62	47	72	70	33	44	48
Ta III w	25	30	28	54	48	34	28	31
Ti III φ	69	64	83	81	76	44	45	-
Ge III nG	12	12	12	16	13	12	10	11
Tr III vTr	12	12	-	10	13	13	10	13
Ta IV d	121	111	-	142	134	78	90	-
Ta IV w	41	38	41	58	40	34	36	-
Ta IV e	55	60	-	75	73	31	34	-
Ti IV φ	102	-	121	71	68	33	36	-
Tr IV vTr	48	49	-	15	13	-	-	-
c_1-c_1	214	200	-	258	222	158	186	198
d_1-d_1	100	101	116	128	121	85	82	108
e_1-e_1	126	116	129	132	151	103	-	101
e_2-e_2	107	70	106	90	127	75	-	58
ad_1	24	22	23	54	57	-	-	-
ad_2	11	10	11	48	55	-	-	-

Ventral side of idiosoma with long setae cp, h_1, h_2, thin setae 1a, 4a, peg-like setae 2a, c3, 3a, 4b, ad_2 and g and tubercle-like setae p_1, p_2, p_3 and h_3. Apodemes I joint medially to form Y – shaped structure, other apodemes as in Fig. 2. Aedagus short. The adanal suckers well developed (Fig. 2).

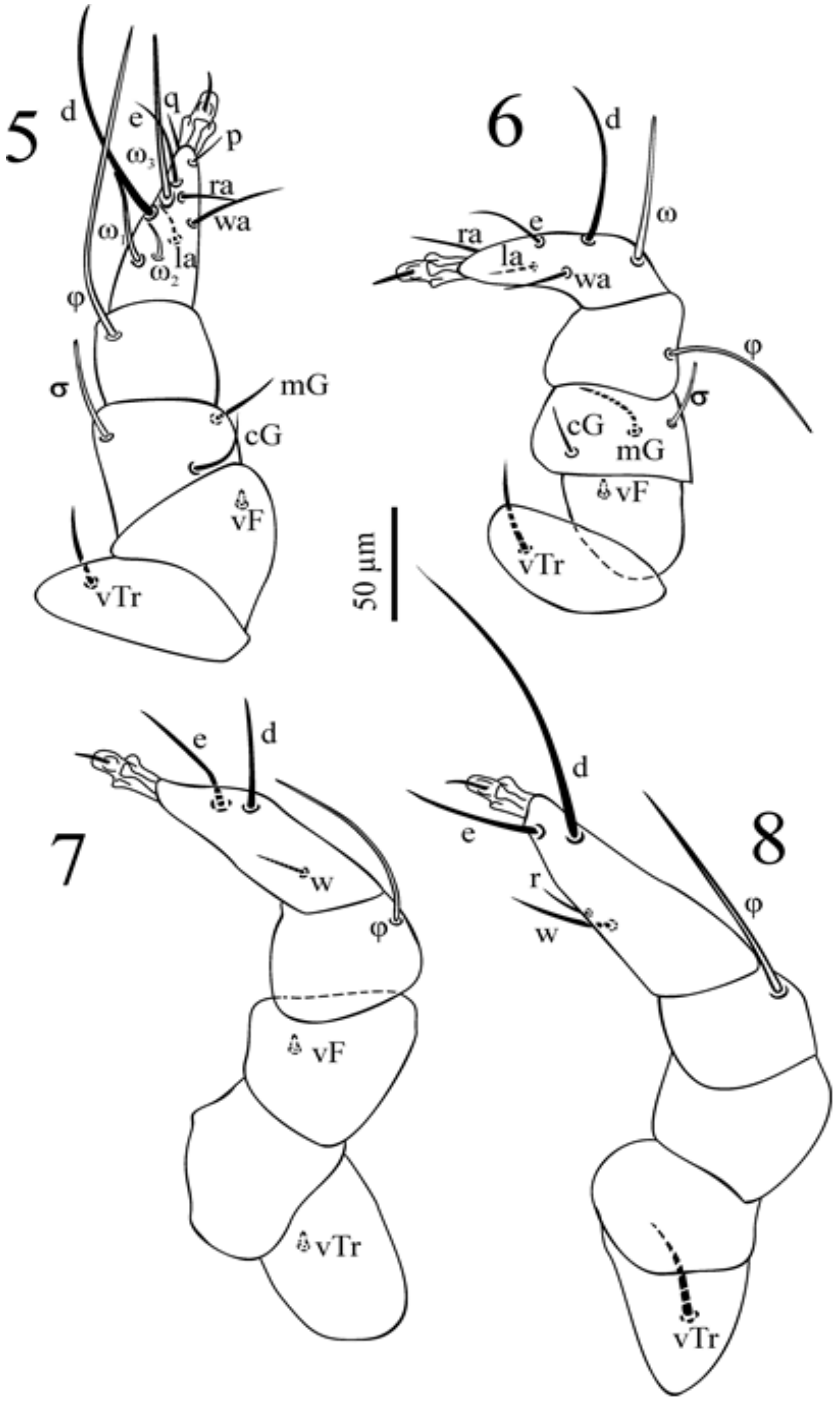


Figures 2. *Cooremania kietaensis* sp. nov. (holotype-male). Ventral view of idiosoma and gnathosoma.

Gnathosoma partly covered by idiosoma, subcapitulum with 2 pairs of nude setae, palps with placed terminally solenidion ω and two pairs of short setae (Fig. 3). Chelicerae with denticles (Fig. 4).

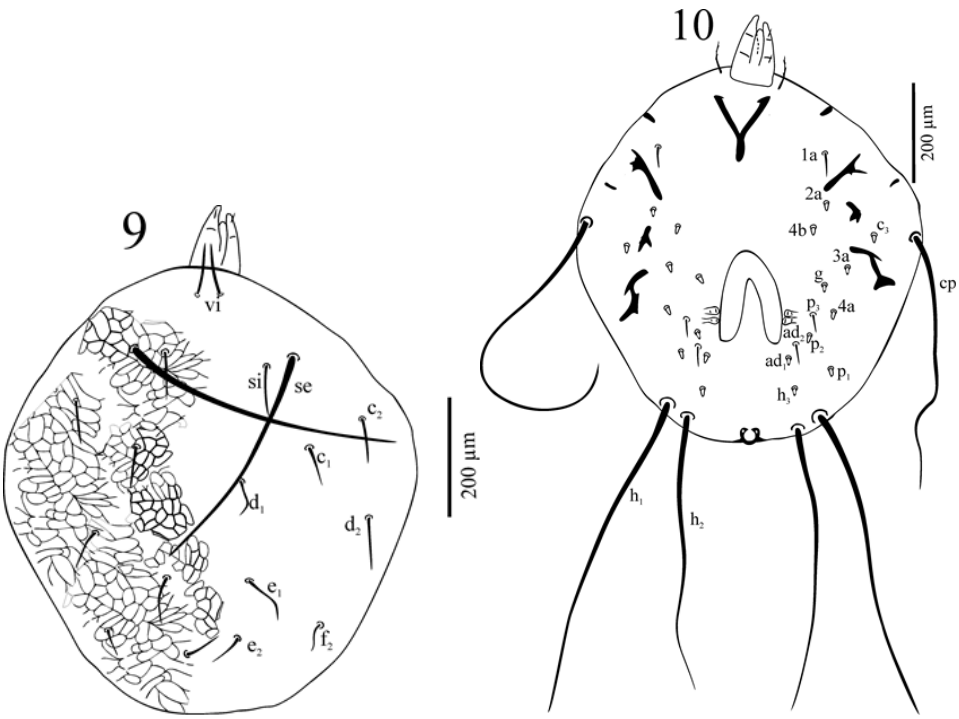


Figures 3-4. *Cooremania kietaensis* sp. nov. (holotype-male). 3. Gnathosoma, 4. Chelicerae.



Figures 5-8. *Cooremania kietaensis* sp. nov. (holotype-male). 5, Leg I, 6, Leg II, 7, Leg III, 8, Leg IV.

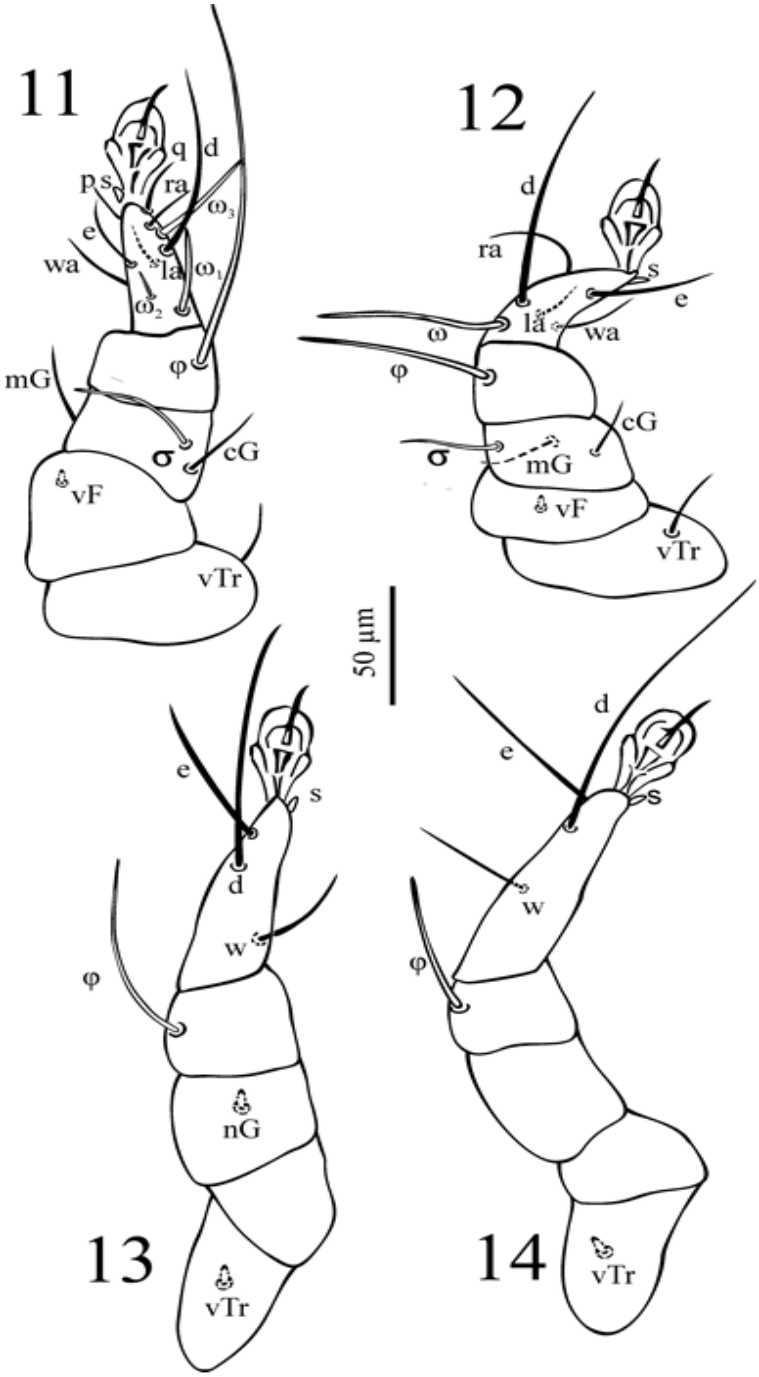
Leg setal formula: Leg I: Ta – ω_1, ω_2, ω_3, d, e, q, p, la, ra, wa, s; Ti – φ, Ge σ, cG, mG; Fe – vF; Tr – vTr (Fig. 5, Table 1). Leg II: Ta – ω, d, e, ra, la, wa, s; Ti – φ, Ge – σ, mG, cG; Fe – vF; Tr – vTr (Fig. 6). Leg III: Ta – d, e, w, s; Ti – φ; Ge – nG; Tr – vTr (Fig. 7). Leg IV: Ta – d, e, w, s; Ti – φ; Tr – vTr (Fig. 8). Leg I: seta d is the longest on Ta, φ is two times longer than φ on Ti II and III and distinctly longer than φ on Ti IV, seta vF is peg-like. Setae vF on Fe II, seta nG on Ge III and Tr III are peg-like.



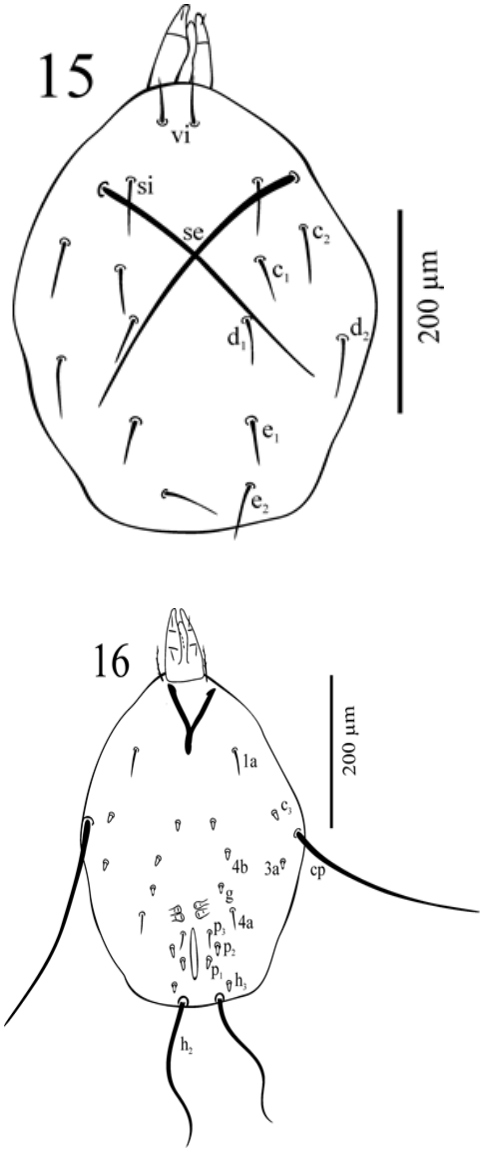
Figures 9-10. *Cooremania kietaensis* sp. nov. (female). 9, Dorsal view of idiosoma and gnathosoma, 10, Ventral view of idiosoma and gnathosoma.

Female (n = 2). The females have longer idiosoma than males, the dorsal setal pattern is identical as in males but all setae are longer, excluding setae h3 and h_2 (one male has this seta longer) (Fig. 9, Table 1).

The whole dorsum is ornamented. Ventral side of idiosoma differs from males in shape of setae in anal and genital region: setae p_1, p_2, p_3 and h_3 are peg-like, in males tubercle-like (Fig. 10). Gnathosoma as in male, partly covered by idiosoma.

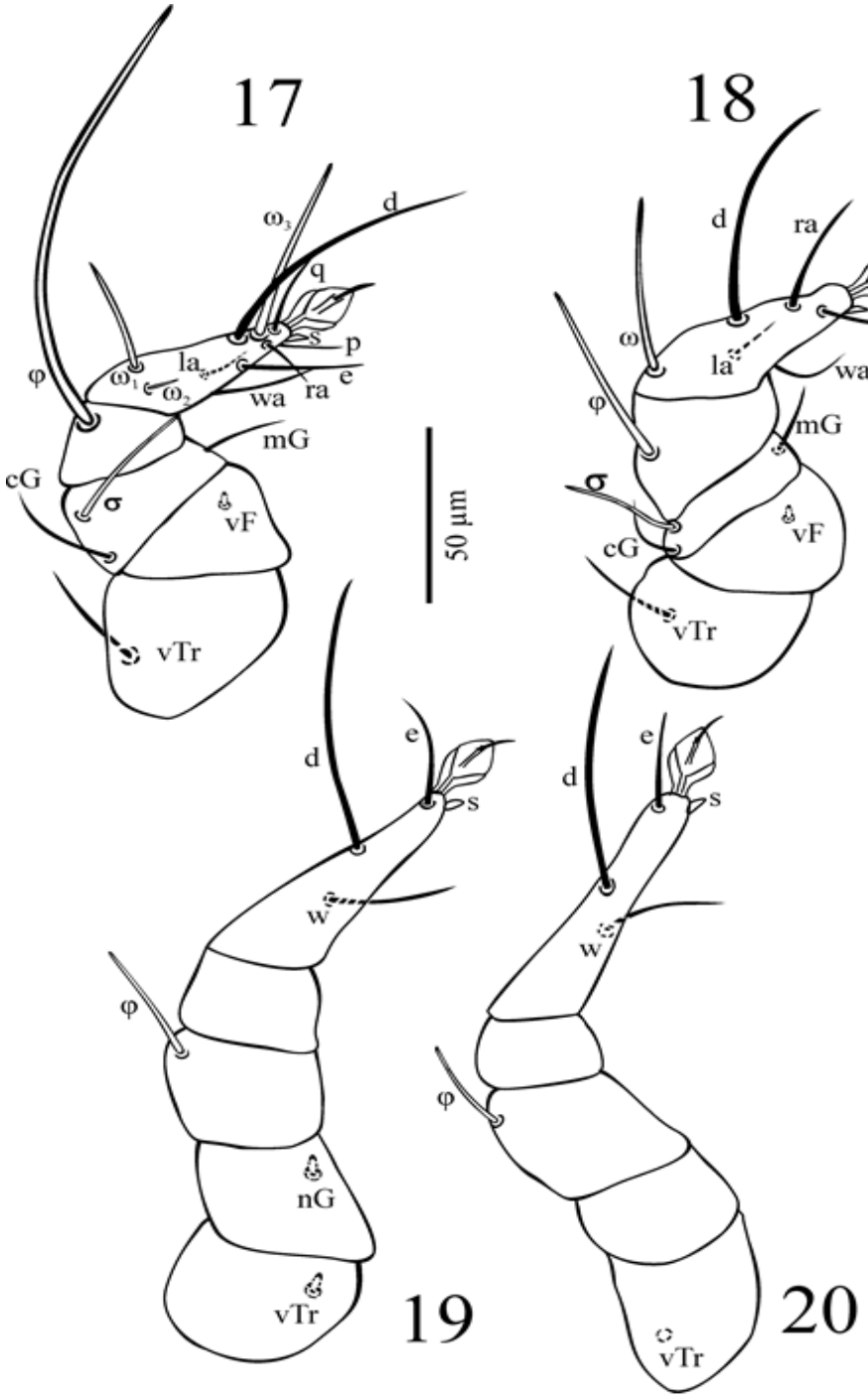


Figures 11-14. *Cooremania kietaensis* sp. nov. (female). 11, Leg I, 12, Leg II, 13, Leg III, 14. Leg IV. Leg setal formula. As in male; the only difference: seta vTr on leg IV is peg-like (Figs. 11-14).



Figures 15-16. *Cooremania kietaensis* sp. nov. (protonymph). 15, Dorsal view of idiosoma and gnathosoma, 16, Ventral view of idiosoma and gnathosoma.

Protonymph (n = 3). Propodosoma with three pairs of setae: long setae se and short setae vi and si. Hysterosoma with seven pairs of setae: long setae h_1 and short setae c_1, c_2, d_1, d_2, e_1 and e_2 (Fig. 15, Table 1) Ventral side of idiosoma with long setae cp and h_2 short and thin setae 1a, 4a and p_3 and peg-like setae c_3, 3a, g, 4b, p_1, p_2 and h_3 (Fig. 16). Gnathosoma as in male, partly covered by idiosoma.



Figures 17-20. *Cooremania kietaensis* sp. nov. (protonymph). 17, Leg I; 18, Leg II; 19, Leg III, 20, Leg IV.

Leg setal formula. Leg I: Ta – ω_1, ω_2, ω_3, d, e, p, la, q, wa, s; Ti – φ; Ge – σ, mG, cG; Fe – vF; Tr —vTr (Fig. 17). Leg II: Ta – ω, d, e, ra, wa, la, s; Ti – φ; Ge – σ, mG, cG; Fe –vF; Tr – vTr (Fig. 18). Leg III: Ta – d, e, w, s; Ti – φ; Ge – nG; Tr – vTr (Fig. 19). Leg IV: Ta – d, e, w, s; Ti – φ; Tr – vTr (Fig. 20). Setae vF on Fe I and Fe II and nG on Ge III nd vTr on Tr III are peg-like.

Measurements are given in Table 1.

Etymology –Named after the type locality.

Type material –Holotype male, seven paratypes: two males and two females and three protonymphs, Kieta, date undetermined, Bougainville Island, Papua New Guinea from undetermined Lucanidae (Insecta; Coleoptera); collector undetermined. All mites are deposited in the Museum of Natural History, Wrocław University, Poland.

Remarks. *Cooremania kietaensis* sp. nov. differs from C. wauensis in males in longer tubercle-like spines with dumpy bases p_1, p_2, p_3, h_3 > 23 μm vs. vs. these setae are peg-like, < 15 μm, setae h1 and se shorter than idiosoma vs. setae h1 and se longer than idiosoma, longer solenidion φ on Ti I (over two times longer than Ta I) vs. solenidion φ on Ti I slightly longer than Ta I, peg-like seta on genu III vs. tubercle-like spine and seven pairs of short setae on hysterosoma vs. six pairs of setae on hysterosoma; in females in posterior margin of idiosoma slightly concave vs. posterior margin almost straight, setae se and h1 shorter than idiosoma vs. setae se and h1 longer than idiosoma, solenidion φ on Ti I over two times longer than Ta I vs. solenidion φ on Ti I shorter than Ta I and hysterosoma with seven pairs of short setae vs. hysterosoma with six pairs of short setae; protonymphs can be not compare on account of laconic Nesbitt description.

REFERENCES

Berlese, A. (1881): Sopra un nuovo generi di Acari parassiti degli insetti. Atti del Reale Istituto Veneto di Scienze, lettere ed arti 7, 747-752.

Bolu, H. (2016): Southeastern Anatolia region insect fauna I (Coleoptera I: Caraboidea; Dytiscoidea; Bostrichoidea; Chrysomeloidea; Cleroidea; Cucujoidea) of Turkey, Agriculture & Forestry, Vol. 62 Issue 4: 125-145, 2016.

Grandjean, F. (1939): La chaetotaxie des pattes chez les Acaridiae. Bulletin de la Societé, Zoologique de France, 64, 50-56.

Griffiths, D. A., Atyeo, W., Norton, R. A., Lynch, C. A. (1990): The idiosomal chaetotaxy of astigmatid mites. Journal of Zoology (London), 220, 1-32.

Haitlinger, R. (1989a): New canestriniid mites (Acari, Astigmata, Canestriniidae) connected with beetles of the subfamilies Dynastinae and Cetoniinae (Insecta, Coleoptera, Scarabaeidae). *Wiadomości Parazytologiczne*, 35, 43-60.

Haitlinger, R. (1989b): New canestriniid mites (Acari, Astigmata, Canestriniidae) associated with Scarabaeidae and Cerambycidae (Insecta, Coleoptera) from the Indo-Australian Region. Wiadomości Parazytologiczne, 35, 585-601.

Haitlinger, R. (1990a): New canestriniid mites (Acari, Astigmata, Canestriniidae) associated with beetles of the families Carabidae, Scarabaeidae, Tenebrionidae and
Passandridae (Insecta, Coleoptera). Annales Zoologici, 43, 111-125.

Haitlinger, R. (1990b): New canestriniid mites (Acari, Astigmata, Canestriniidae) associated with passalid beetles (Insecta, Coleoptera, Passalidae). Polskie Pismo Entomologiczne, 59, 527-589.

Haitlinger, R. (1991): *Rugoniphela* n. gen., *Noemiphela* n. gen. and *Uriophela* n. gen. three new genera of canestriniid mites (Acari, Astigmata) from Asia. Redia, 74, 534-542.

Haitlinger, R. (1993): New genus and two new species of canestriniid mites (Acari, Astigmata) associated with Lucanidae (Insecta, Coleoptera). Mit Zool Mus Berl 69: 339-344.

Haitlinger, R. (1995): New species of mites collected on Tenebrionidae, Scarabaeidae,
Cerambycidae and Lucanidae with the description of new subfamily Vereoxiinae
(Acari, Astigmata, Canestriniidae). Wiadomości Parazytologiczne, 41, 79-91.

Haitlinger, R., Šundić, M. (2016): *Ibizella balearica* n. gen., n. sp. (Astigmata
Canestriniidae) from Balearic Islands, Spain. Redia, XCIX, 75-82. http://dx.doi.org/10.19263/REDIA-99.16.09

Kim, W., Lee, S., Choi, J., Sim, H., Kim, J. (2006): A new canestriniid mite, *Haitlingeria longilobata* n. g., n. sp. (Acari: Astigmata: Canestriniidae), parasitizing *Serodnathus platymelus castanicolor* Motschulsky (Coleoptera: Lucanidae) from South Korea. Systematic Parasitology, 65, 13-18. DOI: 10.1007/s11230-006-9035-9

Kishida, K. (1924): On a new canestriniid mite from Japan. Annot Zool Jap 10: 363-364.

Nesbitt, H. (1976): Five new canestriniids (Acari: Astigmata) from Papau New Guinea. Zoological Journal of the Linnean Society, 58, 219-236.

Norton, R. A. (1998): Morphological evidence for the evolutionary origin of Astigmata
(Acari: Acariformes). Experimental & Applied Acarology, 22, 559-594. DOI: 10.1023/a:1006135509248

Okabe, K., Goka, K. (2008): Potential impacts on Japanese fauna of canestriniid mites (Acari: Astigmata) accidentally introduced with pet lucanid beetles Southeast Asia. Biodiversity and Conservation, 17, 71-81. DOI: 10.1007/s10531-007-9231 1

Summers, F. M., Schuster, R. O. (1981a): *Apalotacaraus*, a new genus of canestriniid mites from passalid beetles. International Journal of Acarology, 7, 17-38.

Summers, F. M., Schuster, R. O. (1981b): Three new genera of canestriniid mites associated with passalid beetles. International Journal of Acarology, 7, 39-46.

Trach, V. A., Khaustov, A. A. (2011) A review of the genus *Coleopterophagus* Berlese, 1882 (Acari: Astigmata: Canestriniidae) of Ukraine. Acarina, 19, 213-230.

Onen, H., Farooq, S., Akyol, N., Ozaslan, C. (2020): Seed dormancy differences among common ragweed (Ambrosia artemisiifolia L.) populations distributed in different climatic regions of Turkey. Agriculture and Forestry, 66 (3): 169-182.

DOI: 10.17707/AgricultForest.66.3.14

Huseyin ONEN, Nihat AKYOL [1], Shahid FAROOQ [2], Cumali OZASLAN [3]

SEED DORMANCY DIFFERENCES AMONG COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) POPULATIONS DISTRIBUTED IN DIFFERENT CLIMATIC REGIONS OF TURKEY

SUMMARY

Common ragweed (*Ambrosia artemisiifolia* L.) is an allergenic invasive weed rapidly expanding its distribution range throughout Europe. Seed dormancy is among the most important seed adaptation traits, helping colonizing plants to avoid adverse environmental conditions. Furthermore, knowledge of seed dormancy enables to predict the seed germination timing of species and enforce specific management practices at suitable times. This study was aimed at determining the seed dormancy differences among different common ragweed populations distributed in two distinct regions of Turkey (i.e., eastern and western parts of the country). The seeds of all populations were extremely dormant (80.50-96.71% dormancy). The populations distributed in western part of the country were more dormant than eastern populations.

Mechanical scarification and cold-wet stratification at 4°C for 2, 3 and 4 weeks were tested to infer their potential in releasing seed dormancy. Cold-wet stratification proved effective in releasing seed dormancy; however, populations differed in the stratification time required for seed dormancy release. Overall, eastern populations became non-dormant with lesser stratification time (i.e., 2 weeks), while western populations took longer stratification time to become completely non-dormant. Mechanical scarification with sandpaper also released seed dormancy and all populations behaved similar for this technique. This knowledge can be used to predict the germination timing of different populations distributed in two distinct regions of the country. Furthermore, the results can also be utilized in implementing effective management strategies at the proper time.

Keywords: Common ragweed, Seed dormancy, Populations, Cold-wet stratification, Mechanical scarification.

[1]Huseyin Onen, (corresponding author: onenhuseyin@gmail.com), Nihat Akyol, Gaziosmanpasa University, Tokat, TURKEY.
[2]Shahid Farooq, Department of Plant Protection, Harran University, Sanlıurfa, TURKEY.
[3]Cumali Ozaslan, Department of Plant Protection, Faculty of Agriculture, Dicle University, Diyarbakır, TURKEY.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:27/06/2020 *Accepted:05/08/2020*

INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is a notorious invasive plant species readily becoming naturalized throughout Europe. The species has successfully colonized almost all of temperate Europe, while its distribution is limited in areas having relatively high temperatures at the start of common ragweed (ragweed hereafter) growing season (Essl et al., 2015). Ragweed negatively affects biodiversity and its pollens are one of the major sources of seasonal allergies for people (Zemmer et al., 2012; Essl et al., 2015; Ozaslan et al., 2016). Moreover, it is also a noxious weed of different arable crops like sunflower, soybean, maize etc. in several regions of the world (Fumanal et al., 2008; Essl et al., 2015; Ozaslan et al., 2016; Önen, 2015). Therefore, ragweed has been considered among the 100 worst invasive species of Europe (DAISIE, 2016). The extensive distribution of the species along roadsides, non-agricultural areas and ruderal habitats is also observed throughout its invasion range (Fumanal et al., 2008; Essl et al., 2015; Ozaslan et al., 2016). Ragweed produces enormous amounts of seeds, which result in rapid range expansion soon after introduction to new geographic regions. Seeds germinate in late spring and germination is stopped with rise in soil temperature (Essl et al., 2015).

Seed dormancy is an important adaptive trait helping plant species to avoid adverse environmental conditions and promotes persistence in the soil seed bank (Willemsen and Rice, 1972). The dispersion of invasive plant species over time can be maximized by seed dormancy (Mandak and Pysek, 2001; Richardson and Kluge, 2008). The persistence of ragweed in soil seed bank requires a dormant period immediately after seed dispersal (Dinelli et al., 2013). Dormant period helps the plant species to avoid adverse environmental conditions and ensures germination in the next growing season if dormancy is broken. It has been well documented that ragweed seeds exhibit both primary and secondary dormancy (Bazzaz, 1970; Willemsen and Rice, 1972; Baskin and Baskin, 1980; Essl et al., 2015). Primary dormancy can be broken by cold-wet stratification at 4°C for 2 weeks (Pickett and Baskin, 1973; Baskin and Baskin, 1980; Fumanal et al., 2006). However, different regional findings suggest the existence of huge variation in ragweed seeds for dormancy and germination among different populations or even within the same population (Willemsen and Rice, 1972; DiTommaso, 2004; Essl et al., 2015). These variations allow invasive plant species like ragweed to persist under a wide range of environmental conditions (Li and Feng, 2009; Eslami, 2011). Seed dormancy helps the colonizing and colonized populations to persist under a specific set of environmental conditions with varying seasonality across different regions. Seed dormancy also excludes the extinction risks and provides opportunities for adaptive divergence.

Different biotic or abiotic factors (external or internal to the seed coat) induce seed dormancy in plant species (Battla and Benech-Arnold, 2007). The seed dormancy status of the seeds continuously fluctuates and reaches to maximum or minimum at different times (Battla et al., 2004). The fluctuations in seed dormancy status hamper the management efforts implemented against

weedy and invasive species through unusual patterns of seedling emergence (Battla and Benech-Arnold, 2007). Hence seed dormancy knowledge is of prime importance for understanding biology and ecology of the species as well as for predicting seedling emergence (Battla and Benech-Arnold, 2007; Gioria and Pyšek, 2017). The development of management strategies against invasive or weedy plant species requires sound knowledge of seed dormancy (Brownsey et al., 2013).

Ragweed is distributed in three distinct regions in Turkey (i.e., Black Sea, Marmara and Inner Anatolia), which have different climates (Onen et al., 2014, 2015, 2016; Ozaslan et al., 2016). The species has started to incur losses to crop production (Onen et al., 2016; Ozaslan et al., 2016) and recently pollens of the species have been observed in various regions of the country (Farooq et al., 2016; Celenk and Malyer, 2017). Moreover, it has been reported that species is tolerant to various abiotic stresses and invasion will be accelerated in the country in the future (Onen et al., 2017). Hence understanding differences among seed dormancy of different populations distributed in distinct regions is essential for development and enforcing site-specific/region-specific management practices.

Although exhaustive work has been done to understand the seed dormancy in ragweed across different geographic regions of the world, variations among different populations have merely been explored at regional scales. Moreover, the studies have largely focused on the cold-wet stratification for 2 weeks only, while different timings have rarely been explored for different populations. Moreover, seed dormancy in ragweed is due to weak embryo and resistance offered by the hard seed coat; however, mechanical scarification has never been explored. This study was therefore designed to explore the seed dormancy differences among ragweed populations distributed in Marmara (Western part) and Black Sea (Eastern part) regions of Turkey. The results will help to understand the biology and ecology of different populations, predict seedling emergence and enforcement of management options at correct/suitable timings for effective management of the species.

MATERIAL AND METHODS

Study area

The study area lies in Turkey, which is a diverse country situated between 25°40' to 44°48'E, and 35°51' to 42°06'N. The total area of the country is 814,578 km^2. Out of the total area, 97 and 3% is located in Asia and Europe, respectively. The country is divided into seven distinct geographical regions namely: Black Sea, Eastern Anatolia, South Eastern Anatolia, Mediterranean, Aegean, Marmara and Inner Anatolia. The ragweed populations included in the study were distributed in Black Sea and Marmara regions of the country. Black Sea region shares a border with Georgia, while Marmara region shares border with Greece and Bulgaria. The travel, transport and trade activities with these countries could transport alien species to the country (Wilson et al., 2016). The country has huge diversity in climate, which makes it suitable for the invasion of non-native

species. Marmara region lies in western side of the country and has a moderate climate with average winter and summer temperature of 4°C and 27°C, respectively. Black Sea region is situated in eastern part of the country and has a wet, warm and humid climate with average winter and summer temperature of 7°C and 23°C, respectively (Sensoy, 2014).

Seed Collection

Ragweed achenes (hereafter seeds) were collected from four different locations, two from Black Sea region (i.e., Samsun and Beşikduzu districts) and two from Marmara region (Keşan and Çorlu districts), which were highly infested with ragweed. Mature seeds were collected from 40-50 mother plants. The seeds spent 3 weeks in the laboratory, then were separated from the trash (leaves, inflorescence etc.) and stored in glass jars. The experiments were conducted immediately after the seed collection to avoid the effects of after-ripening on seed dormancy. The information on climatic features and geographic locations of the populations are presented in Table 1. Similarly, the climatic conditions prevailing at the seed collection sites of ragweed populations during ragweed growth season are provided in Table 2.

Table 1: Geographic information and climatic conditions prevailing at the collection sites of different common ragweed populations in two distinct regions in Turkey

Habitat	Agriculture	Ruderal	Roadside	Agriculture and Roadside
Location	Beşikduzu	Samsun	Keşan	Çorlu
Latitude	41.05	41.38	41.09	41.14
Longitude	39.21	36.21	26.64	27.87
Region	Black Sea	Black Sea	Marmara	Marmara
Aridity Index	1.11	0.89	0.68	0.67
PET (mm)	779.30	813.58	901.24	839.18
Annual rainfall (mm)	865.42	723.60	614.66	563.81
Aridity Class*	Humid - No risk of desertification	Semi humid may be vulnerable to desertification	Semi humid - open to desertification	Semi humid- open to desertification

* Aridity class adopted from Camci et al. (2007), PET= potential evapotranspiration.

Table 2: The climatic conditions prevailing at common ragweed populations during the growth seasons of the species

		Eastern Populations		Western Populations	
Growth phase	**Months**	**Beşikduzu**	**Samsun**	**Keşan**	**Çorlu**
	Minimum Temperature °C				
Germination	May	15.10	15.60	11.60	12.30
	June	19.20	18.70	15.30	16.20
Growth	July	21.70	19.40	17.90	15.80
	August	22.40	21.70	18.80	18.00
Seed set and dispersal	September	19.40	18.90	15.80	14.40
	October	15.40	14.10	11.30	11.20
	Maximum Temperature °C				
Germination	May	19.80	21.20	23.80	24.20
	June	23.30	24.90	28.40	28.90
Growth	July	25.30	27.20	30.90	31.00
	August	25.70	27.30	30.60	30.80
Seed set and dispersal	September	23.40	23.80	26.20	27.00
	October	19.80	18.20	20.40	21.80
	Average Temperature °C				
Germination	May	17.50	16.90	18.70	17.80
	June	20.20	20.80	23.40	22.50
Growth	July	22.50	23.30	25.90	24.90
	August	23.00	23.50	25.70	24.90
Seed set and dispersal	September	20.40	19.80	21.50	21.20
	October	16.60	15.20	17.30	17.50

Presence/Absence of Seed Dormancy

The presence/absence of seed dormancy in the tested populations was determined first. For this purpose, 50 seeds of each population in 5 replicates were placed on two layers of moistened filter paper in each of Petri dishes (5 Petri dishes for each population) having a diameter of 9 cm. The Petri dishes were incubated at 25°C day and night temperature and in complete dark to avoid the effects of light on seed dormancy release. Filter papers were moistened with distilled water according to moisture needs of the seeds. Petri dishes were randomized daily to avoid the effects of temperature on the germination. Germination was observed 30 days after initiation of experiment. The non-germinating seeds were tested for viability using the crush test (Sawma and Mohler, 2002). The final germination percentage was converted to viability adjusted germination (VAG) using the equation 1 (Weller et al., 2016);

$$\text{Viability Adjusted Germination (\%)} = \frac{N_{germ}}{N_{germ} + N_{viable\_non\_germ}} \times 100 \quad (1)$$

Here,

N_{germ}= Total number of germinated seeds,

$N_{viable\_non\_germ}$ = Total number of viable non-germinated seeds

Seed Dormancy Release Experiments

Four different seed dormancy-breaking techniques along with non-treated control were tested to determine their possible role in overcoming dormancy of ragweed seeds and differences among populations arising from distinct climatic regions. The treatments were mechanical scarification with sandpaper, cold-wet stratification at 4°C for 2, 3 and 4 weeks and a non-treated control. Seeds were placed between two layers of moistened filter paper, incubated at 4ºC for 2, 3 or 4 weeks in cold-wet stratification, surface dried and used in the experiments. Seeds were rubbed with sandpaper in mechanical scarification treatment and used in the experiment.

Experimental Layout

The experiment to infer the presence/absence of dormancy was laid in completely randomized design with five replications. Whereas seed dormancy release experiment was laid out in randomized complete block design with split plot arrangements. Ragweed populations were considered as main plots, whereas seed dormancy breaking techniques were randomized in sub-plots.

General experimental procedure

Five replicates of 50 seeds were used for each treatment, and seeds were placed on two layers of moistened filter paper in 9 cm Petri dishes. Petri dishes were moistened with deionized water according to the moisture requirements throughout the experiments. The Petri dishes were incubated at 25°C for 30 days, with 12 hours light and dark period. The light was supplied through cool, white fluorescent lamps in the incubators. The place of Petri dishes was changed daily to avoid any biased effects of the light/dark period on germination. The number of germinated seeds was counted daily and germinated seeds were removed from the Petri dishes. Different germination-related parameters were calculated from the collected data. Mean daily germination (MDG) was calculated by using the equation 2 (Czabator, 1962).

$$\text{Mean Daily Germination} = \frac{\text{Total number of germinated Seeds}}{\text{Total number of days}} \quad (2)$$

Similarly, germination speed (GS) was calculated through using equation 3 (Czabator, 1962)

$$\text{Germination Speed} = \sum(n_1/d_1 + n_2/d_2 + n_3/d_3 + \cdots n_n/d_n) \quad (3)$$

where, n= Total number of germinated seeds; d = Number of days (1. 2. 3……n)

Finally, the final germination percentage at 30 days after initiation of the experiments was calculated as VAG by using equation (1).

Statistical Analysis

The germination data of the dormancy presence/absence were subjected to one-way analysis of variance (ANOVA), whereas a two-way ANOVA was conducted on seed dormancy breaking treatments to determine differences between populations and populations × dormancy breaking techniques'

interactions. Least significant difference test at 99% probability was used to separate the means. All statistical computations were executed on SPSS statistical software version 23 (IBM, 2012). Microsoft Excel program was used for the graphical representation of data and computing standard errors.

RESULTS AND DISCUSSION

Presence/Absence of Seed Dormancy

The tested populations proved highly dormant and significant differences were observed in seed dormancy level of the tested populations (Figure 1). Seeds of the populations collected from Marmara region were more dormant (95.70% and 96.71% dormancy in Keşan and Çorlu populations, respectively) compared with the seeds of populations collected from Black Sea region (Figure 1).

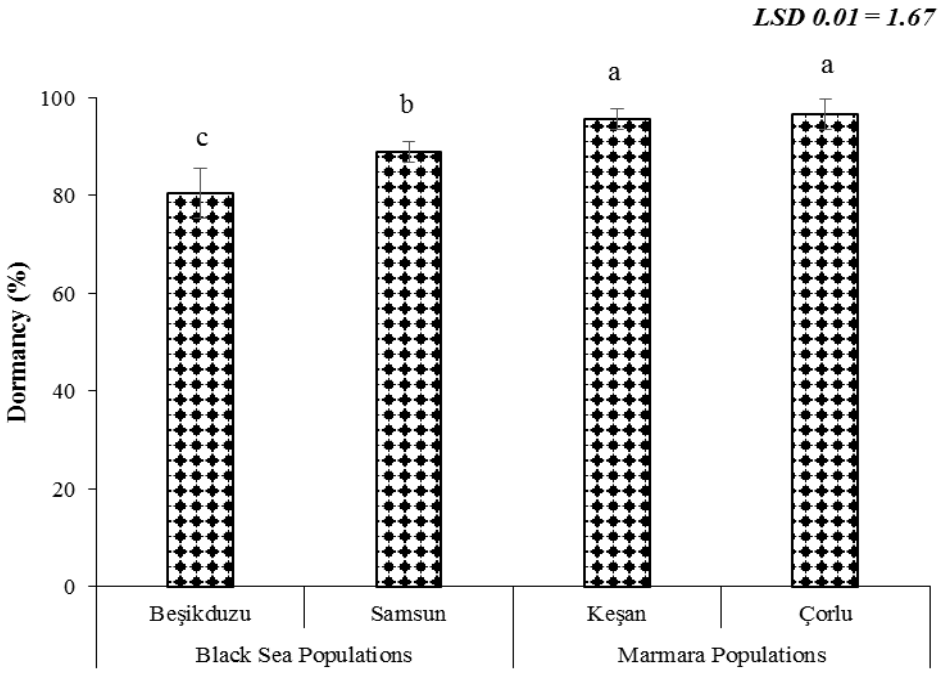


Figure 1: Dormancy level of different populations of common ragweed collected from two distinct climatic regions in Turkey, LSD = least significant difference.

Seed Dormancy Release

Significant differences were observed in ragweed populations, seed dormancy breaking techniques and populations × seed dormancy breaking techniques' interactions for final germination (VAG), time to complete 50% of total germination (T50) and mean daily germination (MDG) (Table 3). Cold-wet stratification stimulated the germination of all tested populations, while seeds of the populations collected from Marmara region required longer stratification time (4 weeks) in this regard (Figure 2). Germination of all populations was also

stimulated by mechanical scarification and all populations responded similarly in this regard (Figure 2). Overall, the highest and lowest final germination was observed in cold-wet stratification for 4 weeks and non-treated seeds, respectively (Figure 2).

Table 3. Two-way Analysis of variance of common ragweed populations, seed dormancy breaking techniques and their interactions for viability-adjusted germination, mean daily germination and time to complete 50% germination of different common ragweed populations collected from two distinct regions in Turkey.

Source		Populations (P)	Techniques (T)	P×T
DF		3	5	15
Sum of Squares	**VAG**	2185.44	73172.55	2015.73
	MDG	0.15	5.08	0.14
	T_{50}	27.26	1345.58	11.87
Mean Square	**VAG**	728.48	14634.51	183.25
	MDG	0.05	1.02	0.01
	T_{50}	9.09	269.12	1.08
F Value	**VAG**	39.72	797.94	9.99
	MDG	39.72	797.94	9.99
	T_{50}	13.67	404.75	1.62
P Value	**VAG**	<0.0001	<0.0001	<0.0001
	MDG	<0.0001	<0.0001	<0.0001
	T_{50}	<0.0001	<0.0001	0.0071

DF= degree of freedom, VAG= Viability adjusted germination (final germination), MDG= Mean daily germination, T_{50}= Time to complete 50% of the total germination, *= significant at 1% level of significance, ns= non-significant

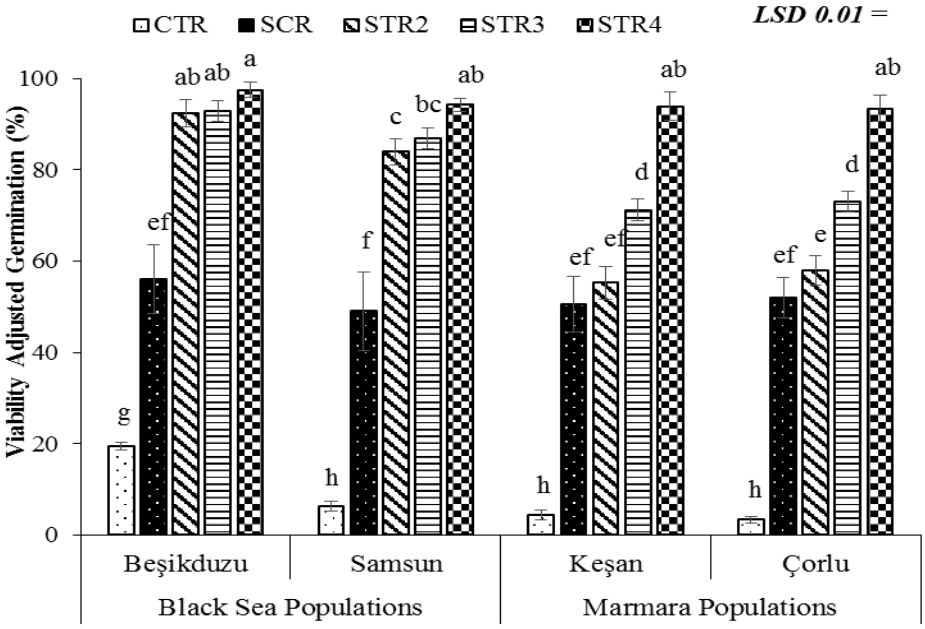


Figure 2: Effect of different seed dormancy breaking techniques on final germination percentage of different populations of common ragweed collected from two distinct climatic regions in Turkey, CTR=non-treated seeds, SCR=mechanical scarification with sandpaper, STR2=cold-wet stratification for 2 weeks, STR3=cold-wet stratification for 3 weeks and STR4=cold-wet stratification for 4 weeks, LSD=least significant difference, The vertical bars show standard error of means and any two means sharing common letter are statistically not different at $p \leq 0.01$.

The increasing duration of cold-wet stratification not only stimulated the germination, but also decreased the time to 50% germination (Figure 3). The highest number of days to T_{50} were taken by control treatment, while the lowest days for T_{50} were taken by cold-wet stratification for 4 weeks. The seeds of the populations collected from Marmara region took longer time to reach T_{50}, whereas seeds of the populations collected from Black Sea region reached to T_{50} in relatively less time for in all seed dormancy breaking techniques (Figure 3).

Mean daily germination was improved by increasing duration of cold-wet stratification in the similar manner as of final germination percentage (Figure 4). The seeds of the populations collected from Marmara region had less MDG compared to the seeds of populations collected from Black Sea region in all seed dormancy breaking techniques except cold-wet stratification for 4 weeks, where all populations behaved similarly (Figure 4). The highest and lowest MDG was observed in cold-wet stratified seeds for 4 weeks of all populations and non-treated seeds, respectively (Figure 4).

Seed dormancy is an important adaptive trait allowing invasive plant species to explore novel environments for range expansion. Significant differences were observed in seed dormancy level of different ragweed populations arising from distinct climatic regions. The differences in seed dormancy level can be attributed to different climatic conditions prevailing in different regions at the time of seed set (Sensoy, 2014). Ecological adaptations of the species for persisting in different geographic regions might be the other reason for differences among seed dormancy level in different populations of the species (Essl et al., 2015).

Cold-wet stratification for different durations was the most effective technique for overcoming seed dormancy of all populations. However, populations significantly differed for stratification time for effective release of seed dormancy. Mechanical scarification also released seed dormancy to significant extent, however it cannot be considered akin to cold-wet stratification. Mechanical scarification probably helped the seeds to overcome the mechanical resistance offered by the seed coat to weak embryo, thus resulted in better germination.

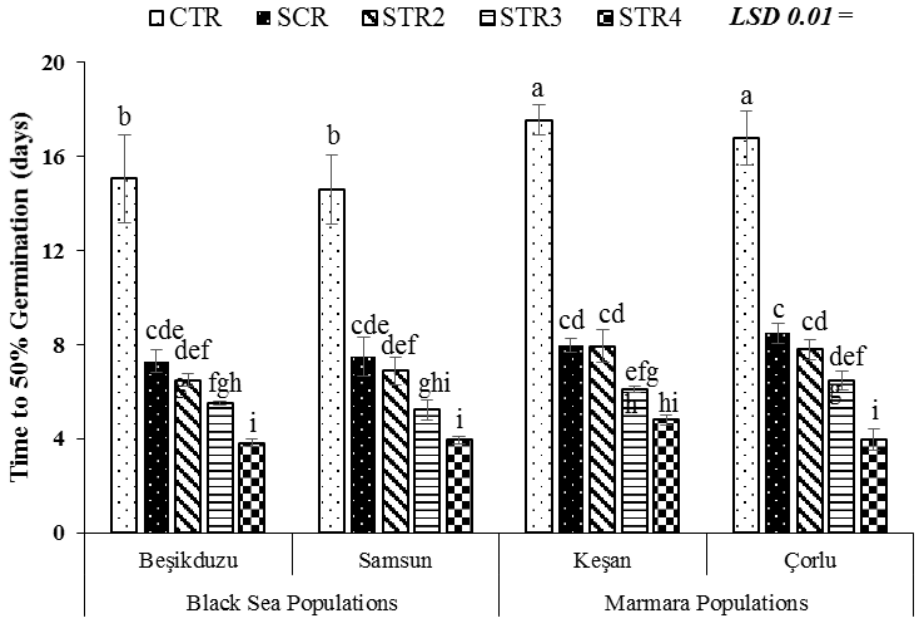


Figure 3: Effect of different seed dormancy breaking techniques on time to 50% germination of different populations of common ragweed collected from two distinct climatic regions in Turkey, CTR=non-treated seeds, SCR=mechanical scarification with sandpaper, STR2=cold-wet stratification for 2 weeks, STR3=cold-wet stratification for 3 weeks and STR4=cold-wet stratification for 4 weeks, LSD=least significant difference, The vertical bars show standard error of means and any two means sharing common letter are statistically not different at $p \leq 0.01$.

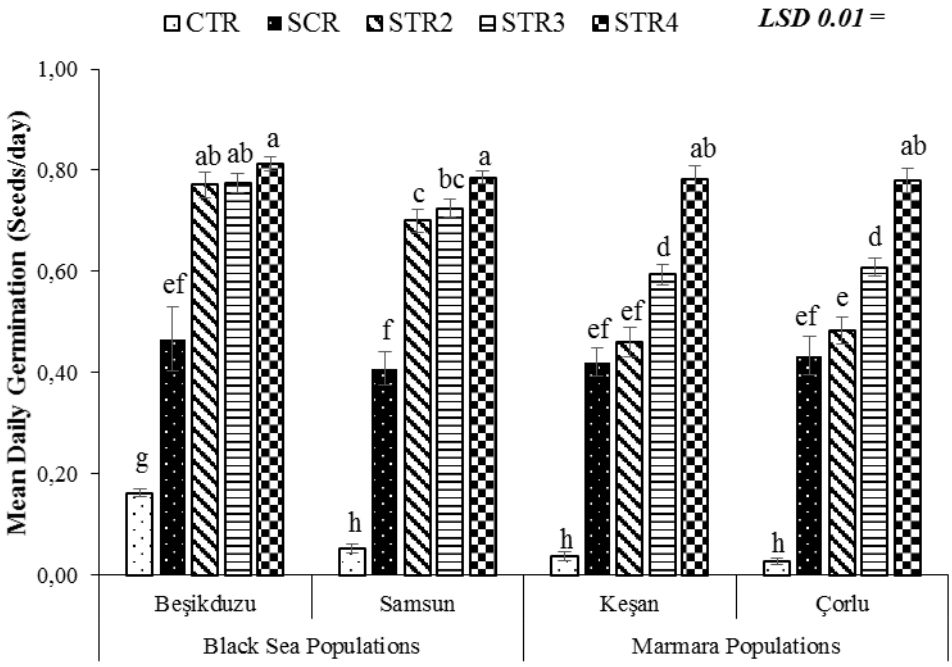


Figure 4: Effect of different seed dormancy breaking techniques on mean daily germination of different populations of common ragweed collected from two distinct climatic regions in Turkey, CTR=non-treated seeds, SCR=mechanical scarification with sandpaper, STR2=cold-wet stratification for 2 weeks, STR3=cold-wet stratification for 3 weeks and STR4=cold-wet stratification for 4 weeks, LSD=least significant difference, The vertical bars show standard error of means and any two means sharing common letter are statistically not different at $p \leq 0.01$

Ragweed can successfully persist in a wide range of habitat and ecological conditions (Essl et al., 2015). Different ragweed populations significantly differed for germination in response to different salinity levels (DiTommaso, 2004). Differences in the germination of different populations of weedy species have been widely cited in different studies (Li and Feng, , 2009; Eslami, 2011). However, some studies also reported that different populations of ragweed show similar germination and dormancy (Dinelli et al., 2013). These findings suggest that ragweed seeds undergo regional adaptations to explore novel environments.

Seed dormancy allows the species to persist in the soil seed bank, and breaking of dormancy while overwintering ensures seed germination in the next growing season. The efficacy of cold-wet stratification in breaking dormancy is directly linked with the germination timing of ragweed at the beginning of the growing season. Several authors have reported that cold stratification for different time periods (2 to 8 weeks) at 4°C effectively breaks the dormancy of ragweed seeds (Willemsen and Rice, 1972; Pickett and Baskin, 1973; Baskin and Baskin,

1980; Fumanal et al., 2006; Essl et al., 2015). Cold-wet scarification requirements for breaking seed dormancy of ragweed correspond well to its distribution range in Turkey as well as across the whole Europe (Essl et al., 2015; Ozaslan et al., 2016). The results also reveal that ragweed requires a specific set of environmental conditions for germination; however, cold-wet stratification for 2 weeks, even did not completely (100% germination) overcome the dormancy and populations required more than 2 weeks of cold-wet stratification for complete release of seed dormancy. The possible reasons of population differences might be the maturity time of the seeds and environmental conditions prevailing at the time of seed maturation. Earlier studies have confirmed that seed collected from different populations of same weeds at different times exhibit significant differences for dormancy (Steadman et al., 2004). Since seeds were collected at same time, the population differences could not be attributed to seed maturation timing.

Ragweed flowers and matures at different times in different geographic regions in Turkey (Author's observation). The species germinates and matures earlier in the Marmara region compared with Black Sea region. Climatic conditions of these regions can be considered as the sole driver of these differences. The differences in the stratification time requirement could be attributed to different maturation timings and ecological adaptations of the species in distinct geographic regions. Stratification requirements could be used to predict the germination timing in different regions and could aid in implementation of management practices.

CONCLUSIONS

The current study concludes that different ragweed populations collected from different climatic regions exhibit significant differences for seed dormancy. Cold-wet scarification successfully overcomes the dormancy, while populations from different climatic regions require different cold-wet stratification duration for complete seed dormancy release. The results of the current study add valuable information on the understanding of ecology of different populations distributed in distinct climatic regions of the country. Moreover, biological changes in the seeds and embryo growth after mechanical scarification need to be explored for better understanding of the phenomenon of dormancy release by mechanical scarification. Nonetheless, a thorough understanding of the germination biology of regional populations is necessary for successful management of ragweed at regional scales.

ACKNOWLEDGEMENTS

The current study was funded by the Scientific and Technological Council of Turkey (TUBITAK) with a Grant Number 113 O 790 as a part of the COST Action (TD 1209 - European Information System for Alien Species).

REFERENCES

Baskin JM, Baskin CC. 1980. Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. Ecology, 61: 475–480.

Batlla D, Benech-Arnold RL. 2007. Predicting changes in dormancy level in weed seed soil banks: implications for weed management. Crop Protection, 26(3): 189–197.

Batlla D, Kruk BC and Benech-Arnold RL. 2004. Modelling changes in dormancy in weed soil seed banks: implications for the prediction of weed emergence. In: Benech-Arnold R.L., Sánchez R.A. (eds.): Handbook of seed physiology. Applications to agriculture. New York: Haworth Press Inc: 245–264.

Bazzaz F. 1970. Secondary dormancy in the seeds of the common ragweed *Ambrosia artemisiifolia*. Bulletin of the Torrey Botanical Club, 302–305.

Brownsey RN, Kyser GB and DiTomaso JM. 2013. Seed and germination biology of *Dittrichia graveolens* (Stinkwort). Invasive Plant Science and Management, 6(3): 371–380.

Camci CS, Karaca A, Haktanır K and Yildiz H. 2007. Global attention to Turkey due to desertification. Environmental Monitoring and Assessment, 128: 489–493.

Celenk S, Malyer H. 2017. The occurrence of *Ambrosia* pollen in the atmosphere of Northwest Turkey: investigation of possible source regions. International Journal of Biometeorology, 1–12.

Czabator FJ. 1962. Germination value: an index combining speed and completeness of pine seed germination. Forest Science, 8: 386–396.

Delivering Alien Invasive Species Inventories for Europe – DAISIE. 2016. 100 of the Worst: http:// www.europe-aliens.org/speciesTheWorst.do (accessed 3.7.3017).

Dinelli G, Marotti I, Catizone P, Bosi S, Tanveer A, Abbas R and Pavlovic D. 2013. Germination ecology of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. biotypes suspected of glyphosate resistance. Open Life Sciences, 8: 286–296.

DiTommaso, A. 2004. Germination behavior of common ragweed (*Ambrosia artemisiifolia*) populations across a range of salinities. Weed Science, 52: 1002–1009.

Ellis R, Roberts E. 1981. The quantification of ageing and survival in orthodox seeds. Seed Science and Technology, 9: 373–409.

Eslami, S. 2011. Comparative germination and emergence ecology of two populations of common lambsquarters (*Chenopodium album*) from Iran and Denmark. Weed science, 59: 90–97.

Essl F, Biró K, Brandes D, Broennimann O, Bullock JM, Chapman DS, Chauvel B, Dullinger S, Fumanal B and Guisan A. 2015. Biological flora of the British Isles: *Ambrosia artemisiifolia*. Journal of Ecology, 103: 1069–1098.

Farooq S, Lommen STE, Hallmann CA and Onen H. 2016. Seed and pollen production variations among Ragweed populations in Black Sea region. In: Book of Abstracts. Turkey 6th Plant Protection Congress with International Participation, Konya, Turkey, September 5–8, 2016.

Fumanal B, Girod C, Fried G, Bretagnolle F and Chauvel B. 2008. Can the large ecological amplitude of *Ambrosia artemisiifolia* explain its invasive success in France? Weed Research, 48: 349–359.

Fumanal B, Plenchette C, Chauvel B and Bretagnolle F. 2006. Which role can arbuscular mycorrhizal fungi play in the facilitation of *Ambrosia artemisiifolia* L. invasion in France? Mycorrhiza, 17: 25–35.

Gioria M, Pyšek P. 2017. Early bird catches the worm: germination as a critical step in plant invasion. Bioogical Invasions, 19(4): 1055–1080.

IBM Corporation. (2012): IBM SPSS statistics for Windows, version 21.0. IBM Corporation Armonk, NY.

Li YP, Feng YL. 2009. Differences in seed morphometric and germination traits of crofton weed (*Eupatorium adenophorum*) from different elevations. Weed Science, 57: 26–30.

Mandak B, Pyšek P. 2001. Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. Journal of Ecology, 89: 159–165.

Onen H, 2015. Invasive plants catalogue of Turkey. Ankara, Turkey: Ministry of Food, Agriculture and Livestock. Ankara Turkey. ISBN: 978-605-9175-05-0. In Turkish.

Onen H, Gunal H and Ozcan S. 2014. The Black Sea highway: the route of common ragweed (*Ambrosia artemisiifolia* L.) invasion in Turkey. In: Book of Abstracts: 8th International Conference on Biological Invasions from understanding to action, Antalya, Turkey, November 3–8, 2014.

Onen H, Ozaslan C and Akyol N 2015. *Ambrosia artemisiifolia* L. In: Onen H. (eds): Invasive Plants Catalogue of Turkey. ISBN: 978-605-9175-05-0. In Turkish.

Onen H, Farooq S, Gunal H and Ozaslan C. 2016. Common ragweed invasion in Turkey: What we have learnt so far? In: Book of Abstracts. Turkey 6th Plant Protection Congress with International Participation, Konya, Turkey, September 5–8, 2016.

Onen H, Farooq S, Gunal H, Ozaslan C and Erdem H. 2017. Higher tolerance to abiotic stresses and soil types may accelerate common ragweed (*Ambrosia artemisiifolia*) Invasion. Weed Science, 65(1): 115–127.

Ozaslan C, Onen H, Farooq S, Gunal H and Akyol N. 2016. Common ragweed: An emerging threat for sunflower production and human health in Turkey. Weed Biology and Management, 1: 42–55.

Pickett ST, Baskin JM. 1973. The role of temperature and light in the germination behavior of *Ambrosia artemisiifolia*. Bulletin of the Torrey Botanical Club, 100: 165–170.

Richardson DM, Kluge RL. 2008. Seed banks of invasive Australian Acacia species in South Africa: role in invasiveness and options for management. Perspectives in Plant Ecology, Evolution and Systematics, 10: 161–177.

Sawma JT, Mohler CL. 2002. Evaluating seed viability by an unimbibed seed crush test in comparison with the tetrazolium test 1. Weed Technology, 16: 781–786.

Sensoy, S. 2014. Climate of Turkey: http://topraksuenerji.org/Climate_of_turkey.pdf (accessed 3.7.2017).

Steadman KJ, Ellery AJ, Chapman R, Moore A and Turner NC. 2004. Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). Crop and Pasture Science, 55: 1047–1057.

Weller S, Florentine S, Sillitoe J, Grech C and McLaren D. 2016. An investigation of the effects of stage of ensilage on *Nassella neesiana* seeds, for reducing seed viability and injury to livestock. Scientific reports, 6. doi:10.1038/srep22345.

Willemsen RW, Rice EL. 1972. Mechanism of seed dormancy in *Ambrosia artemisiifolia*. American Journal of Botany, 59(3): 248–257.

Wilson JRU, García-Díaz P, Cassey P, Richardson DM, Pyšek P and Blackburn TM. 2016. Biological invasions and natural colonisations are different: the need for invasion science. NeoBiota, 31: 87–98.

Zemmer F, Karaca F and Ozkaragoz F. 2012. Ragweed pollen observed in Turkey: detection of sources using back trajectory models. Science of the Total Environment, 430, 101–108.

Uttaruk, Y., Laosuwan, T. (2020): Methods of estımatıon for above ground carbon stock ın Nongbua-nonmee communıty forest, Maha Sarakham Provınce, Thaıland. Agriculture and Forestry, 66 (3): 183-195

DOI: 10.17707/AgricultForest.66.3.15

Yannawut UTTARUK[1], Teerawong LAOSUWAN[2]

METHODS OF ESTIMATION FOR ABOVE GROUND CARBON STOCK IN NONGBUA-NONMEE COMMUNITY FOREST, MAHA SARAKHAM PROVINCE, THAILAND

SUMMARY

The climate of the world today has changed greatly. This is mainly due to human activity causing large emissions of carbon dioxide (CO_2) from the reserve into the atmosphere, which is the main cause of global warming. This research methodology comprised of training for people in the community and local government sector, so they acquired knowledge and understanding of the causes and consequences of global warming. The people will also learn the adjustment toward weather atmosphere, the method in relieving global warming issues with the potential of the community forest as well as tree measurement techniques. The research method was based from the analysis of the carbon stock from the community forest from 3 different methods; which are 1) method of tree measurement by farmers in the community 2) Measurement, Reporting and Verification (MRV) online method, and 3) the application of Geoinformatics Technology (GIT). The research results found that 50 participants in the training session possessed over 80 percent of understanding toward causes and consequences from global warming, adjustment toward the change of weather atmosphere, and way to relieve the severity of global warming issue with a potential of community forest. Participants were also able to learn tree measurement techniques as well as able to record results. The results from the data analysis from the field survey, MRV online tool, and Geoinformatics Technology found that the community forest can complete the process of carbon stock that is equivalent to 5,256.66 tCO_2e, 5,061.32 tCO_2e, 5,058.01 tCO_2e respectively.

Keywords: *Community forest, Carbon stock, Ecological structure, Remote Sensing, Geoinformatics technology, MRV.*

[1]Yannawut Uttaruk, (corresponding author: teerawong@msu.ac.th) Department of Biology, Faculty of Science, Mahasarakham University, Khamrieng, Katarawichai, Maha Sarakham, 44150, THAILAND.

[2]Teerawong Laosuwan, Department of Physics, Faculty of Science, Mahasarakham University, Khamrieng, Katarawichai, Maha Sarakham, 44150, THAILAND.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:16/05/2020 *Accepted:30/08/2020*

INTRODUCTION

When radiations from the sun pierce through the atmosphere to the earth surface, it will enter the earth crust where the atmosphere will reflect some parts to space. The Greenhouse Gas (GHG) will act as an absorbent of the heat radiation, so the weather on the earth surface is warm and suitable for the existence of the living organisms. Thus, without these types of gases, the average temperature of the earth surface will decrease down to 0°F (approximately -17.78°C). These GHGs in an appropriate amount will help average the heat on the earth surface to around 59°F (about 15°C) (Gore, 2007). Thus, the high amount of Greenhouse Gases (GHG) that derived from human actions will increase the severity of the greenhouse effect, which results in the average temperature of the earth to increase drastically. This will lead to a tremendous change in the weather atmosphere. Significant GHGs are including Carbon dioxide (CO_2), Methane (CH_4), Nitrous oxide (N_2O), Hydrofluorocarbons (HFC), Perfluorinated compounds (PFC), and Nitrogen trifluoride (NF_3) (Uttaruk and Laosuwan, 2016). The CO_2 is a GHG that we give importance the most as it has up to 80 percent ratio of GHG that was released from human activities. Examples of human activities were fuel combustion from factories, deforestation to build resident or for agricultural purposes. The deforestation takes up almost 20 percent for the CO_2 emission to the atmosphere (Uttaruk and Laosuwan, 2018). This was because trees and forests have excellent quality in the absorbent of CO_2 (Senpaseuth et al., 2009). Therefore, the decreased number of forest area will result in an increasing amount of CO_2 (Spracklen et al., 2015; Ounkerd et al., 2015; Bradford and Bell, 2017). At present; CO_2 sequestration can be done in two methods which are 1) direct storage which is a method to prevent the diffusion of CO_2 to the atmosphere, and 2) the indirect storage. This method is considered the best method for the carbon stock, which was to store it in trees and forests as trees and forests are the best sources for the carbon stock (IPCC, 2018).

Therefore, forest pays an essential role in the sequestration and emission of CO_2. The sequestration or absorption of CO_2 through the process of photosynthesis will be completed when tree brings about CO_2 to use in absorbing foods, production, and creation of biomass (Cairns et al., 1997; Mekuria et al., 2015; Sisay et al., 2017). The forest had three significant effects on global warming; which are, 1) forest can absorb CO_2 from the atmosphere and help to maintain and stabilize the earth's temperature. The second effect was the forest help release vapor into the atmosphere and increase moist. The third effect was that the forest covered the entire area of land from the sun, which also helps to reduce the heat in the earth surface (Laosuwan and Uttaruk, 2016a; Rotjanakusol and Laosuwan, 2018). Thus, international scientific research that was published in "National Science Foundation Report" from the United States stated that growing of trees, increase numbers of forest area, sustainable agriculture, and conservation of wetlands as well as a better management of soil all can help effectively reduce the severity of the global warming. Growing of forests will help reduce up to 37 percent of 11.3 billion tons of CO_2 emission by 2030 or

equal to the fuel combustion. This would be enough to maintain the earth surface not to exceed 2°C (The Nature Conservancy, 2019). For Thailand, in 1973 the area of the forest was about 221,707 km^2 or approximately 43.21% of the area in Thailand. However, in 2017, the forestry area has tremendously reduced to about 163,981.28 km^2 or equivalent to 31.68 % (Royal Forest Department Thailand, 2019). The number of forest intrusion increased drastically every year. This was due to the significant change in the economic structures from agricultural to industrial; this resulted in the deterioration of forest and a drastic reduction in the number of forest (Royal Forest Department Thailand, 2016).

The management of the community forest is another trend that people in the community pay attention to more than ever before (Laosuwan et al., 2016b). The people also began hosting activities for the conservation and preservation of the forest (Poungngamchuen, 2013). Thus, each local area tends to have format and method on the management of the community forest differently, but less likely to succeed. This was because it was done only done through the participation of the people (Dyer et al., 2014; Samek et al., 2014). There was no cooperation from the government sector as much as needed. Therefore, the involvement from the people and the cooperation from government sectors as well as educational institutes are significant to the management of the community forest (Samek et al., 2011; Husseini et al., 2016; Tadesse et al., 2017). The objective of this research project was to development methods of estimation for above ground carbon stock in Nongbua-nonmee community forest, Bua Kho Sub-district, Mueang District, Maha Sarakham province, Thailand. Thus to raise awareness within the community on the causes and consequences from the global warming, adjustment toward the changes of weather atmosphere, and method to relieve the severity of global warming issues with potential of the community forest.

MATERIAL AND METHODS

To make it concise and easy to understand, this research illustrated operational methods into steps as follow:

Transfer of Knowledge

The research transferred the knowledge through lecturer from the researcher on topics of 1) causes and consequences from global warming 2) the adjustment toward the change of the weather atmosphere and 3) method on relieving the severity of global warming from the community forest. Thus, the researcher has evaluated the results of participants in the training on the three topics above with questionnaires.

Operational Training

The researcher hosted an operational training on the carbon stock measurement techniques in biomass from trees within the study area through the placement of sample permanent plot in a size of 20x20 m^2 for the total of 15 plot (Figure 1). Thus, participants acquired knowledge and understanding on 1) survey planning process 2) tree measurement techniques based on the theory as

well as the use of Global Positioning System (GPS) to record results in the sample permanent plot and 3) method in recording surveyed data and store in a systematical manner.

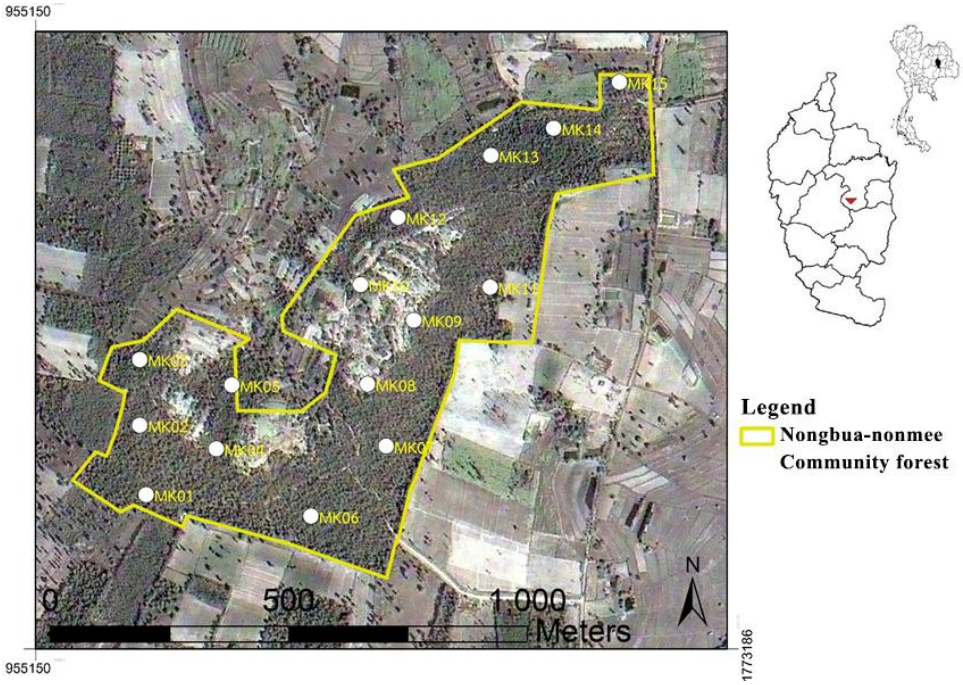


Figure 1. Nongbua-nonmee community forest

Data survey of ecological structures

This research has surveyed the ecological structures to evaluate the diversity of plant species in the community forest from the sample permanent plot in a size of 20x20 m for the total of 15 plots. After that, the results were taken to analyze the plant communities through the evaluation of Importance Value Index (IVI) (Curtis and McIntosh, 1950; Mishra, 1968; Muller-Dombois and Ellenberg, 1974). The aim of this was to analyze the status and importance or dominant species that affect the amount of carbon stock within the forestry area.

Data analysis

There were three operational methods for the carbon stock analysis as follow: 1) The analysis of the carbon stock was from the tree measurements during the field survey by the people in the community. The researcher will use the results to calculate the amount of carbon stock in the community forest with the allometric equation for the mixed forest and deciduous forest as developed by Ogawa et al. (1965). The results will be displayed as seen in equation 1.

$$W_s = 0.0396\left(D^2H\right)^{(0.9326)}$$
$$W_b = 0.003487\left(D^2H\right)^{(1.0270)} \quad (1)$$
$$W_l = (0.28/\left(W_s + W_b + 0.025\right)^{-1}$$
$$ABG = \left(Ws + Wb + Wl\right)$$

Where;

W_s is the weight of the stem (kg); W_b is the weight of branches (kg); W_l is the weight of leaves (kg); D is the diameter at breast height (cm); H is the tree height (m)

2) The analysis on the carbon stock from MRV online tool. The MRV tool was developed by Michigan State University, USA (http://mrv.carbon2 markets.org/cas/login/) and can analyze the carbon stock with all types of trees.

3) The analysis of carbon stock with Geoinformatics Technology (GIT). The researcher used data from Landsat 8 satellite and analyzed as follow:

(1) Adjust the value of digital number to top of atmosphere reflectance (Uttaruk and Laosuwan, 2016),

(2) Analyze the vegetation with MSAVI-2 (Qi et al., 1994; Laosuwan and Uttaruk, 2014) with equation 2,

(3) The results based on the calculation from step2 will be analyzed for the Fractional Vegetation Cover (FVC) (Gitelson et al., 2002; Zhang et al., 2019) value with equation 3, and

(4) The analyzed result from step 3 will be using to find the correlation with the amount of surveyed carbon from the field data.

$$\text{MSAVI-2} = \frac{2*NIR+1-\sqrt{(2*NIR+1)^2-8*(NIR-RED)}}{2} \quad (2)$$

Where;

RED is the red band reflectance of Landsat 8; NIR is the Near Infrared band reflectance of Landsat 8

$$FVC = \frac{(VI-VI_{open})}{\left(VI_{canopy}-VI_{open}\right)} \times 100 \quad (3)$$

Where;

FVC is the tree canopy fractional cover; VI is the vegetation index; VI_{open} is the vegetation index of open areas;

VI_{canopy} is the vegetation index of tree canopy

RESULTS AND DISCUSSION

Results from the Transfer of Knowledge

There were *50 people in the area surrounding the community forest were interested in participation* of the research project. From the evaluation of

questionnaire, it found that participants acquired understandings of topic (1), (2), and (3) for 81 percent. About 8 percent understand certain topics and another 11 percent did not provide opinions in the questionnaire. Regarding the acknowledgment of changes toward the weather atmosphere – In general, the people will receive the news on the changes in weather atmosphere from a different form of media. However, the people still lack awareness on knowledge and consequences of the moves toward weather atmosphere. From the project, it was found that the group that was engaged in the transfer of knowledge tended to respond and become aware of the consequences than those who never receive knowledge transfer. This was because the people still lack the basic understanding on the carbon cycle in daily life.

Results from the Operational Training

The results for the evaluation of knowledge and ability for the tree measurement in 50 participants found that those that received operational training were able to measure the growth (diameter) and height of the tree. Participants were also able to record measured data into the record form. After the categorization, it was found that 37 percent of training participants were able to measure tree growth with tools. Additionally, 50 percent of participants were able to use estimation method; the rest of 13 percent did not provide any opinions. For the height measurement of tree, it was found that 24 percent of training participants used tools to measure, 68 percent used estimation, and the rest of 8 percent did not provide any opinions.

Results from the Data Survey of the Ecological Structures

The results from data survey of the ecological structure to estimate the diversification of species in the community forest found 33 different plant species from 23 families. Five plant species that were mostly found including:

1) *Dipterocarpus tuberculatus* Roxb. DIPTEROCARPA CEAE,

2) *Shorea obtusa* Wall. ex Blume: DIPTEROCARPACEAE,

3) *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C. Nielsen : LEGUMINOSAE-MIMOSOIDEAE,

4) *Dipterocarpus obtusifolius* Teijsm ex Miq.: DIPTEROCARPACEAE,

5) *Catunaregam tomentosa* (Blume ex DC.) Tirveng.: RUBIACEAE.

After an analysis of the plant communities through the Importance Value Index (IVI) evaluation (Table 1) which consisted of Relative Density (RD), Relative Frequency (RF), and Relative Dominance (RDo), it was found that five dominant species were

1) *Dipterocarpus tuberculatus* Roxb.

2) *Shorea obtusa* Wall. ex Blume

3) *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C. Nielsen

4) *Dipterocarpus obtusifolius* Teijsm ex Miq.

5) *Catunaregam tomentosa* (Blume ex DC.) Tirveng

Table 1. Importance Value Index (IVI)

No.	Scientific name	RD	RF	RDo	IVI
1	*Dipterocarpus tuberculatus* Roxb.	27.344	10.924	37.494	75.762
2	*Shorea obtusa* Wall. ex Blume	19.271	9.244	10.796	39.311
3	*Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C. Nielsen	10.417	9.244	7.668	27.329
4	*Dipterocarpus obtusifolius* Teijsm ex Miq.	8.854	4.202	11.148	24.204
5	*Catunaregam tomentosa* (Blume ex DC.) Tirveng.	4.688	8.403	3.673	16.764
6	*Buchanania lanzan* Spreng.	2.344	5.882	2.630	10.856
7	*Canarium subulatum* Guillaumin	2.604	4.202	2.657	9.463
8	*Memecylon edule* Roxb.	2.344	3.361	3.631	9.336
9	*Sindora siamensis* Teijsm. & Miq.	2.083	4.202	2.635	8.920
10	*Buchanania* sp.	2.344	2.521	2.185	7.050
11	*Heterophragma sulfureum* Kurz	1.302	3.361	2.259	6.923
12	*Lannea coromandelica* (Houtt.) Merr.	1.563	3.361	1.875	6.799
13	*Terminalia alata* Heyne ex Roth	2.865	2.521	1.210	6.596
14	*Gluta laccifera* (Pierre) Ding Hou	1.823	2.521	1.251	5.595
15	*Strychnos nux-blanda* A.W.Hill	0.781	2.521	2.086	5.388
16	*Pterocarpus macrocarpus* Kurz	1.302	2.521	0.949	4.773
17	*Casearia grewiifolia* Vent. var. *grewiifolia*	1.042	2.521	0.539	4.102
18	*Diospyros ehretioides* Wall. ex G.Don	1.042	2.521	0.459	4.021
19	*Parinari anamense* Hance	1.042	1.681	0.947	3.670
20	*Aporosa ficifolia* Baill.	0.521	1.681	1.422	3.623
21	*Ellipanthus tomentosus* Kurz var. *tomentosus*	0.781	1.681	0.377	2.839
22	*Litsea glutinosa* (Lour.) C.B.Rob.	0.521	1.681	0.344	2.545
23	*Aporosa villosa* (Wall. ex Lindl.) Baill.	0.260	0.840	0.568	1.668
24	*Diospyros ferra* (Willd.) Bakh. var. *ferra*	0.521	0.840	0.209	1.570
25	*Millingtonia hortensis* L.f.	0.260	0.840	0.185	1.286
26	*Ochna integerrima* (Lour.) Merr.	0.260	0.840	0.124	1.225
27	*Grewia eriocarpa* Juss.	0.260	0.840	0.117	1.218
28	*Cratoxylum cochinchinense* (Lour.) Blume	0.260	0.840	0.117	1.218
29	*Mitragyna hirsuta* Havil.	0.260	0.840	0.098	1.199
30	*Terminalia chebula* Retz. var. *chebula*	0.260	0.840	0.086	1.187
31	*Lophopetalum wallichii* Kurz	0.260	0.840	0.086	1.187
32	*Dillenia obovata* (Blume) Hoogland	0.260	0.840	0.086	1.187
33	*Peltophorum dasyrachis* (Miq.) Kurz	0.260	0.840	0.086	1.187
	Total	**100**	**100**	**100**	**300**

This also corresponded to plant types that were commonly found from the survey. In addition, the analysis of species diversity index was completed to identify the diversity of plant types by the Shannon-Wiener Index (Shannon and Weaver, 1949), it was found that in the 316 rai (1 hectare=6.25 rai) of community forest, there was 30 vegetation species in 33 families. The species diversity index was equivalent to 2.514 from the highest possible index of 3.497. It showed that the evenness of the dispersion index was equivalent to 0.719 as shown in Table 2.

Table 2. The species diversity index

Community forest	Area (rai)	Number type	Number of families	H'	H_{max}	J
Nongbua-nonmee	316	33	23	2.514	3.497	0.719

Data analysis results:

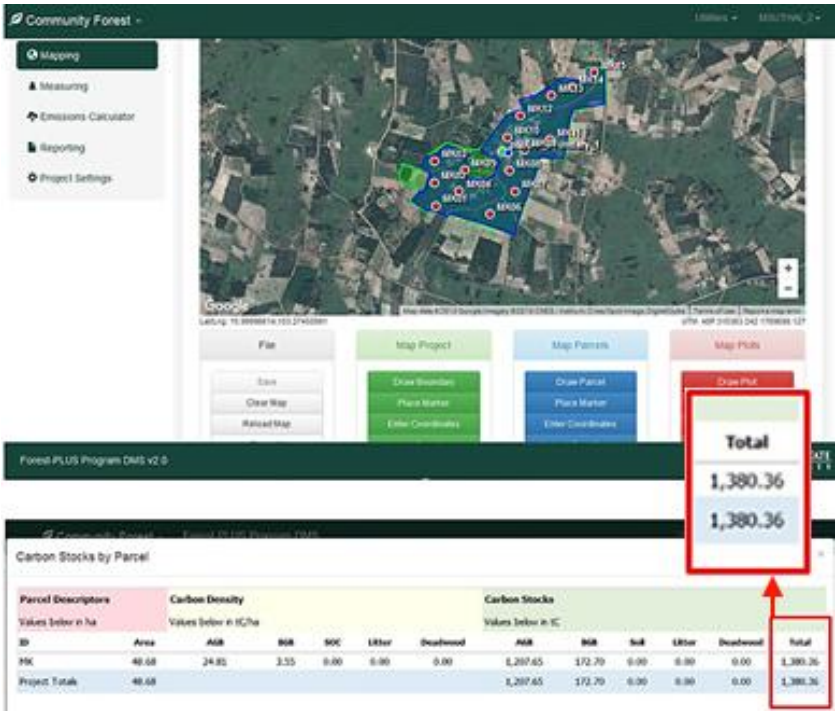


Figure 2. The carbon stock from MRV tool

1) The researcher has taken results from the tree measurements during the field survey of people in the community to calculate the amount of carbon stock in the community forest with allometric equation. The data analysis results found that 316 rai of community forest was able to complete the carbon stock that was equal to 1,433.64 tCO_2 or equivalent to5,256.66 tCO_2e.

2) Login to the MRV system, it required

(1) Indicate qualities of the project based on the types and climate zone as well as contact detail for authorized person for the data management of the project.

(2) It required area scope for the study of the project, area of the educational institution, and location data on sample permanent lot used for the measurement in the field survey based on GPS to display the mapping data in the project.

(3) It required data analysis method, the analysis result of the carbon stock from MRV tool (Figure. 2) found that 316 rai of the community forest was able to held carbon stock that was equal to 1,380.36 tCO_2 or equivalent to 5,061.32 tCO_2e.

3) The results from the data analysis with geoinformatics technology in this research project can be, as seen in Figure. 3.

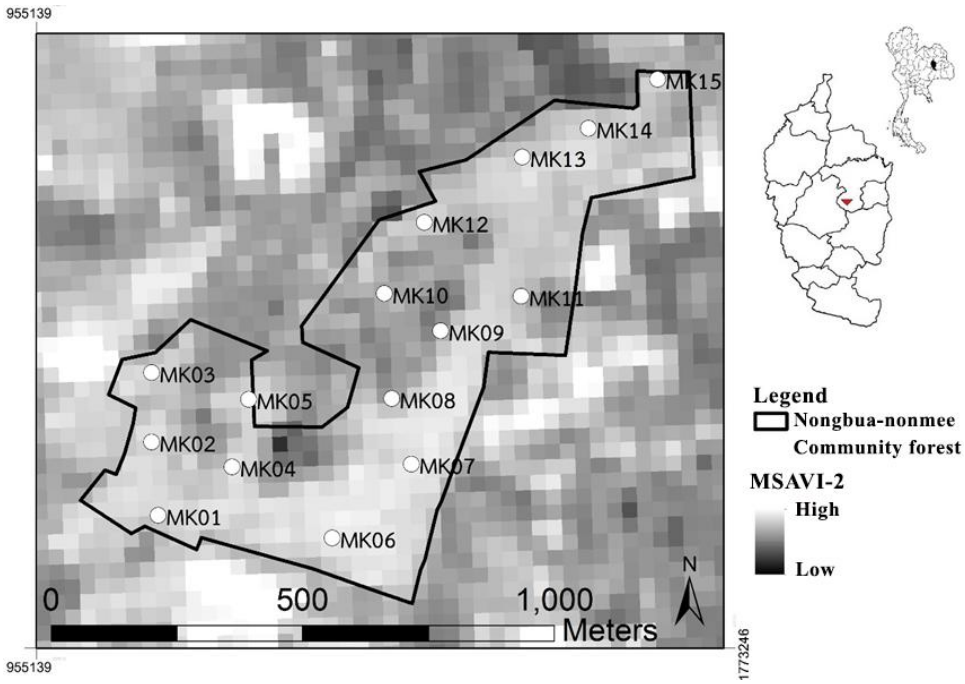


Figure 3. MSAVI-2 analysis

The FVC value was made to find the relation with the amount of carbon stock from the field survey, which results in the relation equation of $y=1.1952e^{0.0402x}$ and the coefficient of determination of $r^2=0.890$. The equation was taken to calculate for the amount of carbon stock in 316 rai of the community forest, the amount of carbon stock was equal to 1,379.46 tCO_2 or equivalent to 5,058.01 tCO_2e.

The results from the data analysis from the field survey, MRV online tool, and Geoinformatics Technology found that the Nongbua-nonmee community forest can complete the process of carbon stock that is equivalent to 5,256.66 tCO_2e, 5,061.32 tCO_2e, 5,058.01 tCO_2e respectively.

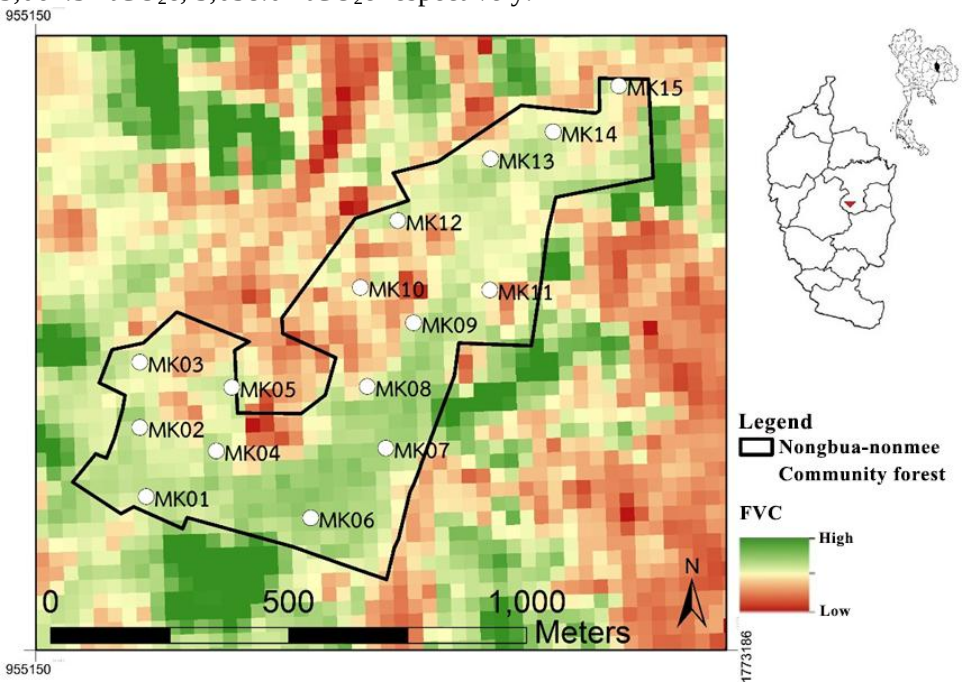


Figure 4. FVC analysis

In addition, the study also found that the result was in the same direction with other research such as: Estimation of carbon stock in south of western Carpathians from Moldova Noua forest district using G.I.S. data from managements plans (Chivulescu and Schiteanu, 2017), Improvement the evapotranspiration estimates using Remote Sensing Techniques and Fuzzy Regression (Parviz, 2018), and Community forest for global warming mitigation: the technique for estimation of biomass and above ground carbon storage using remote sensing method (Uttaruk and Laosuwan, 2018).

CONCLUSIONS

This research project was to propose methods of estimation for above ground carbon stock in Nongbua-nonmee community forest, Bua Kho Sub-district, Mueang District, Maha Sarakham province, Thailand. Considering the study, the estimation of carbon stock requires no field study in all areas which will reduce the expense of field study and also reduce the time of researching while the data will be up-to-date and respond the demand of data in the abreast time. For this research project, it used advanced technology and applied to evaluate the carbon stock in the community forest that was convenient, quick, and

reliable. The project used MRV online tool and Geoinformatics technology. Thus, the estimated result of the carbon stock was taken to test for the accuracy with Pair Sample T-test, it was found that all three methods have a statistically significant of 95%. Each of the methods can be chosen to evaluate the carbon stock for the community forest.

ACKNOWLEDGEMENTS

This research was financially supported by National Research Council of Thailand (NRCT).

REFERENCES

Bradford, J.B., and Bell D.M. 2017. A window of opportunity for climate-change adaptation: easing tree mortality by reducing forest basal area. Frontiers in Ecology and the Environment, 15(1), 11-17.

Cairns, M.A., Winjum, J.K., Phillips, D.L., Kolchugina, T.P., and Vinson, T.S. 1997. Terrestrial carbon dynamics: Case studies in the former Soviet Union, the conterminous United States, Mexico and Brazil. Mitigation and Adaptation Strategies for Global Change, 1(4), 363-383.

Chivulescu S., Schiteanu I., (2017) Estimation of carbon stock in south of western Carpathians from Moldova Noua forest district using G.I.S. data from managements plans, Agriculture and Forestry, 63 (3): 39-46.

Curtis, J.T., and McIntosh, R. P. 1950.The interrelations of certain analytic and synthetic of phytosociological characters. Ecology, 31(3), 434–455.

Dyer, J., Stringer, L.C., Dougill, A.J., Leventon, J., Nshimbi, M., Chama, F., Kafwifwi, A., Muledi, J.I., Kaumbu, J.-M.K., Falcao, M., Muhorro, S., Munyemba, F., Kalaba, G.M., Syampungani. S. 2014. Assessing participatory practices in community-based natural resource management: Experiences in community engagement from southern Africa. Journal of Environmental Management, 137, 137-145.

Gitelson, A.A., Kaufman, Y.J., Stark, R., Rundquist, D. 2002. Novel algorithms for remote estimation of vegetation fraction. Remote Sensing of Environment, 80 (1), 76–87.

Gore, Al. 2007. An Inconvenient Truth: The Crisis of Global Warming. Penguin Books USA, New York, 192 p.

Husseini, R., Kendie, S.B., and Agbesinyale, P. 2016. Community participation in the management of forest reserves in the Northern Region of Ghana. International Journal of Sustainable Development & World Ecology, 23(3), 245-256.

IPCC. 2018. Global warming of 1.5°C. https://report.ipcc. ch/sr15/pdf/sr15_spm_ final.pdf (Accessed: 15 November 2019).

Laosuwan, T., Uttaruk, Y. 2014. Estimating Tree Biomass via Remote Sensing, MSAVI 2, and Fractional Cover Model. IETE Technical Review, 31(5), 362-368.

Laosuwan, T., Uttaruk, Y. 2016a. Application of Geo-informatics and Vegetation Indices to Estimate Above-ground Carbon stock. Studia Universitatis Vasile Goldis Arad, Seria Stiintele Vietii, 26(4), 449-454.

Laosuwan, T., Uttaruk, Y., Rotjanakusol, T., Laosuwan, N., Dumrongsukit, S., and Pattaaumpan., K. 2016b. Public Participation in the Management of Community Forests: A Case Study on Community Forests at Koke Kralong Pong Dang, Nong Reua, Na Chuak, Maha Sarakham. Hatyai Academic Journal, 14(2), 149-169.

Mekuria, W., Langan, S., Johnston, R., Belay, B., Amare, D., Gashaw, T., Desta, G., Noble, A., and Wale, A. 2015. Restoring aboveground carbon and biodiversity: a case study from the Nile basin, Ethiopia. Forest Science and Technology, 11(2), 86-96.

Mishra, R. 1968., Ecology Work Book. Oxford and IBH Publishing Co, Calcutta, 244 p.

Muller-Dombois, D., Ellenberg, H. 1974. Aims and Methods of Vegetation Ecology. John Wiley & Sons. New York, 547 p.

Ogawa, H., Yoda, K., Ogino, K., and Kira, T. 1965. Comparative ecological study on three main types of forest vegetation in Thailand. II. Plant biomass. Nature and Life in Southeast Asia, 4, 49-80.

Ounkerd, K., Sunthornhao, P., and Puangchit, L. 2015. Valuation of Carbon Stock in Trees at Khao Wong Community Forest, Chaiyaphum Province. Thai Journal of Forestry, 34 (1), 29-38.

Parviz, L. (2018): Improvement the evapotranspiration estimates using Remote Sensing techniques and Fuzzy Regression. Agriculture and Forestry, 64 (2): 121-136.

Poungngamchuen, J., Supa-udomlerk Trirat, S., and Rungkawat, N. 2013. Participatory Development of the People in Community Forest Management Model: A Case Study of Baan Tha Pha Pao, Tha Pra Duk Sub-district, Maetha District, Lampun Province. KMUTT Research and Development Journal, 36(2), 215-234.

Qi, J., Chehbouni, A., Huete, A.R., Kerr, Y.H. 1994. Modified Soil Adjusted Vegetation Index (MSAVI). Remote Sensing of Environment, 48(2), 119-126.

Rotjanakusol T., Laosuwan T. 2018. Remote Sensing Based Drought Monitoring in The Middle-Part of Northeast Region of Thailand. Studia Universitatis Vasile Goldis Arad, Seria Stiintele Vietii, 28(1), 14-21.

Royal Forest Department Thailand. 2016. Forest Department Strategy 2016-2021. http://forestinfo.forest.go.th/Content/file/forest%20strategy%2059_64. pdf. (Accessed: 11 December 2016).

Royal Forest Department Thailand. 2019. Forest Information. http://forestinfo. forest.go.th/Content.aspx?id=72/ (Accessed: 02 October 2019).

Samek, J. H., Skole, D. L., Klinhom, U., Butthep. C., Navanugraha, C., Uttaruk, P., and Laosuwan, T. 2011. Inpang Carbon Bank in Northeast Thailand: A community effort in carbon trading from agroforestry projects. In Carbon stock Potential of Agroforestry Systems, 8, 263-280.

Samek, J. H., Skole, D. L., Klinhom, U., Laosuwan, T., Uttaruk, P., Butthep. C. 2014. Smallholders in Thailand and REDD+ and FLEGT linkages. ETFRN news 55: Linking FLEGT and REDD+ to Improve Forest Governance, 55, 134-144.

Senpaseuth, P., Navanugraha, C., and Pattanakiat, S. 2009. The Estimation of Carbon Storage in Dry Evergreen and Dry Dipterocarp Forests in Sang Khom District, Nong Khai Province, Thailand. Environment and Natural Resources Journal, 7(2), 1-11.

Shannon, C. E., and Weaver, W. The Mathematical Theory of Communication. Urbana, IL, The University of Illinois Press, 1949,132 p.

Sisay, K., Thurnher, C., Belay, B., Lindner, G., Hasenauer, H. (2017). Volume and Carbon Estimates for the Forest Area of the Amhara Region in Northwestern Ethiopia. Forests, 8 (4), 122.

Spracklen, B.D., Kalamandeen, M., Galbraith, D., Gloor, E., and Spracklen, D.V. 2015. A Global Analysis of Deforestation in Moist Tropical Forest Protected Areas. PLoS ONE, 10(12), e0143886.

Tadesse, S., Woldetsadik, M., Senbeta, F. 2017. Forest users' level of participation in a participatory forest management program in southwestern Ethiopia. Forest Science and Technology, 13(4), 164-173.

The Nature Conservancy. 2019. Nature's Make or Break Potential for Climate Change.https://www.nature.org/en-us/what-we-do/our-insights/perspectivets/ natures-make-or-break-potential-for-climate-change/(Accessed: 15 Feb 2019).

Uttaruk, Y., and Laosuwan, T. 2016. Remote sensing based vegetation indices for estimating above ground carbon stock in orchards. Agriculture and Forestry, 62 (4), 193-201.

Uttaruk, Y., and Laosuwan, T. 2018. Community forest for global warming mitigation: the technique for estimation of biomass and above ground carbon storage using remote sensing method. Agriculture and Forestry, 64(3), 47-57.

Zhang, S., Chen, H., Fu, Y., Niu, H., Yang, Y., Zhang, B. 2019. Fractional Vegetation Cover Estimation of Different Vegetation Types in the Qaidam Basin", Sustainability, 11(3), 864.

El Bılalı, H., Bassole, I. H. N., Dambo, L., Berjan, S. (2020): Climate change and food security. Agriculture and Forestry, 66 (3): 197-210.

DOI: 10.17707/AgricultForest.66.3.16

Hamid EL BILALI[1]*, Imaël Henri Nestor BASSOLE[2], Lawali DAMBO[3], Sinisa BERJAN[4]

CLIMATE CHANGE AND FOOD SECURITY

SUMMARY

Climate change is one of the most pressing challenges facing humanity in the Anthropocene era. It is widely admitted that climate change will have far-reaching impacts including on food security. Therefore, this review paper analyses the multifaceted relations between climate change and food security. In particular, the paper explores the impacts of climate change on the four dimensions of food security (i.e. food availability, food access, food utilization, stability). It draws upon a review of scholarly literature indexed in the Web of Science. The analysis of the literature shows that there is a dual relationship between climate change and food security; on the one hand, climate change affects all the dimensions of food security and, on the other hand, the quest for food security has implications in terms of climate change. As for food availability and supply, climate change is widely believed to reduce crop yields and livestock productivity especially in the countries of the Global South. Effects on food production and availability as well as the impacts of extreme climate events affect both food physical and economic accessibility. The changes in production systems induced by climate change may induce changes in dietary patterns and food utilization. Climate change will also affect the stability and resilience of food systems with consequences in terms of long-term food security. Moreover, the quest for food security, through agricultural intensification and agricultural land expansion, increases greenhouse gas emissions from deforestation and land use changes. The intricate relation between climate change and food security makes the case for integrated policies that maximise co-benefits while addressing trade-offs. That is fundamental to make sure that 'climate action' will not jeopardize the achievement of 'zero hunger' and vice-versa.

Keywords: Climate change, food security, food availability, food access, food utilisation, food system, agriculture.

[1]Hamid El Bilali, (corresponding author: elbilali@iamb.it), International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Valenzano (Bari), ITALY.

[2]Imaël Henri Nestor Bassole, Joseph KI Zerbo University of Ouagadougou, Ouagadougou, BURKINA FASO.

[3]Lawali Dambo, Abdou Moumouni University of Niamey, Niamey, NIGER.

[4]Sinisa Berjan, Faculty of Agriculture, University of East Sarajevo, East Sarajevo, BOSNIA AND HERZEGOVINA.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Received:20/06/2020 *Accepted:01/09/2020*

INTRODUCTION

According to the Food and Agriculture Organisation of the United Nations (FAO, 2009), "Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life". Food security is built on four pillars (Committee on World Food Security, 2012; Ericksen, 2008; FAO et al., 2013; United Nations System High Level Task Force on Global Food Security, 2011): food availability (i.e. sufficient quantities of food produced and supplied on a consistent basis); food access (i.e. physical access and affordability); food utilisation (i.e. proper use of food based on basic nutrition knowledge); and stability in food availability, access and utilization. The concept of food security (FS) has been central in the discussion on sustainable development. Indeed, the first Millennium Development Goal (MDG) aimed to "Eradicate extreme poverty and hunger" (United Nations, 2015a). More recently, from 2016 on, the second Sustainable Development Goal (SDG) "Zero Hunger" aims to "End hunger, achieve food security and improved nutrition and promote sustainable agriculture" (United Nations, 2015b). However, despite efforts made over the last decades, food insecurity is still a pressing issue in many countries, especially developing ones. In fact, the report on the State of Food Security and Nutrition in the World 2019 (FAO et al., 2019) shows that more than 820 million people were hungry in 2018, especially in Africa, Latin America and Asia. Nevertheless, it was estimated that over 2 billion people do not have a good FS status, including 8% of the population of developed countries (e.g. North America and Europe), when also considering people affected by moderate levels of food insecurity (FAO et al., 2019).

Food insecurity is a symptom of the dysfunction of the global food system (Capone et al., 2016; Capone et al., 2018; El Bilali et al., 2018; El Bilali, 2018; El Bilali, 2019), which is under the unprecedented confluence of various pressures (FAO, 2014) such as climate change (FAO, 2016). Indeed, FAO (2016) puts that "Through its impacts on agriculture, climate change will have negative effects on food security in all of its dimensions […] While food security will be affected through other channels – for example, by extreme weather events that reduce urban dwellers' incomes and thus access to food – agriculture is a key channel through which climate change affects food security" (p. 8). Climate change affects the natural resources (e.g. water, land) used in agricultural production (Cadro et al., 2019; Čustović et al. 2012; Simunic et al., 2019). Beyond its impacts on agriculture and food security, climate change (CC) is one of the most pressing challenges facing humanity (Intergovernmental Panel on Climate Change, 2012; Steffen et al., 2015; United Nations, 2015b). It is central in the ongoing debate on sustainable development. Indeed, the SDG 13 "Climate action" aims to "Take urgent action to combat climate change and its impacts" (United Nations, 2015b). Furthermore, CC represents a threat towards the achievement of different SDGs such as SDG2 "Zero hunger" (Mugambiwa & Tirivangasi, 2017). In this context, the present paper aims to analyse the

multifaceted relations between CC and food security. In particular, it explores the impacts of CC on the four dimensions of food security. The paper draws upon a review of all documents indexed in the Web of Science viz. Science Citation Index Expanded (SCIE), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (A&HCI), Conference Proceedings Citation Index - Science (CPCI), and Emerging Sources Citation Index (ESCI). A search was performed in June 2020 using the Title-Abs-Key search query {climate change} AND {food security}.

EFFECTS OF CLIMATE CHANGE ON FOOD SECURITY DIMENSIONS

Climate change and food availability

Climate change affects food availability through its adverse impacts on crop yields, and fish and livestock productivity, especially in Sub-Saharan Africa (SSA) and South Asia, where most of the food insecure people live. Indeed, Zougmoré et al. (2018) suggest that "[…] without appropriate interventions, climate change and variability will affect agricultural yields, food security and add to the presently unacceptable levels of poverty in sub-Saharan Africa". The effects of CC will be particularly severe in regions where agriculture is predominantly rain-fed such as in SSA, which makes it highly vulnerable to climate fluctuations and droughts (Adhikari et al., 2015; Cooper et al., 2008; Muchuru & Nhamo, 2019). Webersik and Wilson (2009) put that "African economies are closely linked to natural resources and rely heavily on agriculture, largely rain fed […]. It is predicted that Africa will be particularly vulnerable to climate change and climate variability associated with biodiversity loss, food insecurity, water scarcity and an increase in drought frequency" (p. 400). CC will reduce the yields and productions of the main staple crops such as rice (Akinbile et al., 2015; van Oort & Zwart, 2018), wheat (Trnka et al., 2019) and maize (Davenport et al., 2018; Freduah et al., 2019; Murray-Tortarolo et al., 2018; Waha et al., 2013), CC is also predicted to affect livestock productivity (Godber & Wall, 2014; Mare et al., 2018; Naah & Braun, 2019). Ramasamy (2010) put that "Rising temperatures, more intense droughts, floods, and greater weather variability all mean productivity losses to crops and livestock" (p. 185). Some studies also report an increase in the incidence of animal diseases that is attributed to CC (Hussain et al., 2016). Furthermore, the impacts of CC on crops and livestock are strongly linked; for instance, the decrease in the growth of forages reduce livestock weight gains (Butt et al., 2005). Further studies also analyse the potential adverse effects of CC on fisheries (Ding et al., 2017; Lam et al., 2012; Lauria et al., 2018). Referring to the Ganges-Brahmaputra-Meghna (Bangladesh/India) and the Volta (Ghana) deltas, Lauria et al. (2018) suggest that "changes in temperature and primary production could reduce fish productivity and fisheries income especially in the Volta and Bangladesh deltas" (p. 1566). The impacts of CC on fisheries, and consequently fish-based economies, are expected to be particularly high in Small Island Developing States (SIDS) (Dey et al., 2016; Moustache, 2017).

Most of the scholarly literature focuses on CC damages on agriculture thus food availability; nevertheless, Hasegawa et al. (2018) point out that stringent CC mitigation measures can also increase the risk of food insecurity in SSA. Hence, the challenge ahead is how to balance socio-economic development (including food security) and environmental conservation (e.g. CC mitigation) (Rudi et al., 2012). Moreover, some scholars assume that the so-called 'carbon fertilisation' may offset somehow the impact of CC on crop yields. In fact, Roudier et al. (2011) highlight "the pivotal role that the carbon fertilization effect may have on the sign and amplitude of change in crop yields" (p. 1073). Likewise, Sultan and Gaetani (2016) point out that "a robust evidence of yield loss in West Africa emerges. This yield loss is mainly driven by increased mean temperature while potential wetter or drier conditions as well as elevated CO2 concentrations can modulate this effect". Moreover, global warming could expand both the agricultural area and growing season in some northern countries such as Russia (Kiselev et al., 2013), with positive effects on agriculture production.

Yields and productivity declines can have serious implications also for the other FS dimensions, especially food access and food utilisation. For instance, Solaymani (2018) shows a "negative impact of rainfall-temperature variability […] on food availability and access to food due to a reduction in the supply of agricultural products, a commodity inflation pressure and a reduction in household income" (p. 1575) in Malaysia. Wheeler and von Braun (2013) argue that "food access and utilization will be affected indirectly via collateral effects on household and individual incomes, and food utilization could be impaired by loss of access to drinking water and damage to health" (p. 508).

Climate change and food access

Climate change will reduce food access through negative impacts on both food prices and rural livelihoods. Food supply shortfalls, due to negative impacts of CC on yields and production, would increase food prices. Increases in food prices would affect millions of low-income people that live in areas that are already affected by high rates of hunger and poverty, such as South Asia (Bandara & Cai, 2014) and SSA (Tamako & Thamaga-Chitja, 2017). Tamako and Thamaga-Chitja (2017) suggest that "Sub-Saharan Africa is faced with a range of climate risks, which include rapid and uncertain changes in rainfall and temperature patterns that threaten food production, and could lead to an increase in food prices and food insecurity" (p. 16). Poor urban and rural people, most of whom are net food buyers and already spend a high share of their income on food, would find it more difficult to meet their dietary needs, which increase the risk of food insecurity and hunger. The situation is particularly alarming for populations that depend on agriculture for their livelihoods and income, especially small-scale farmers in the Global South (Descheemaeker et al., 2016; García de Jalón et al., 2018; Williams et al., 2018; Wood et al., 2014). In this regard, Williams et al. (2018) put that "The impacts of changing climate on agriculture have consequences on livelihoods and food security. Smallholder

farmers, who have heterogeneous farming systems and limited resources, compounded with multiple risks, are greatly affected". Akinseye et al. (2020) argue that "Climate variability and change will have far reaching consequences for smallholder farmers in sub-Saharan Africa, the majority of whom depend on agriculture for their livelihoods". Indeed, rural populations will suffer from the increase of food prices as well as the negative impacts of CC on their sources of income and livelihoods strategies relating to agriculture. Indeed, agricultural production loss implies the loss of income for farmers and of revenues for countries (Sultan et al., 2019) as agriculture is an important source of income and livelihoods in rural areas and a significant contributor to the national gross domestic product in developing countries (Diao et al., 2007). Decrease of production will be often combined with the loss of livelihoods assets due to the increase of the intensity and frequency of climate-related disasters (Habiba et al., 2016; Poudel et al., 2017). Indeed, severe droughts or floods can dramatically reduce incomes and cause asset losses thus eroding income earning capacity. Poor rural people (e.g. smallholders, agricultural workers) are particularly vulnerable to disaster impacts.

The impacts of CC will be also high on food importing countries as food exports might be reduced or banned in case of extreme climate events. For instance, Qureshi et al. (2013) show that CC will reduce Australian food exports and put that "Despite its relatively small contribution to total global food supply, Australia's contribution to international trade in wheat, meat and dairy products is substantial and could affect global food prices" (136), especially in South and South-East Asia, which already suffer from food insecurity. While most of the focus is on the impacts of CC on economic accessibility (i.e. affordability), CC will also impact food physical accessibility in remote regions. For instance, referring to the Himalayan region, Hussain et al. (2016) report that "After hazards, households face transitory food insecurity owing to damage to their local food systems and livelihood sources, and constrained food supply from other areas" (p. 921). Gregory et al. (2005) highlight the diversity of pathways through which CC affects FS and argue that "Climate change may affect food systems in several ways ranging from direct effects on crop production (e.g. changes in rainfall leading to drought or flooding, or warmer or cooler temperatures leading to changes in the length of growing season), to changes in markets, food prices and supply chain infrastructure" (p. 2139).

Climate change and food utilisation

Climate variability will change food utilisation with impacts on the nutrition status of the populations, especially poor and vulnerable people. For instance, higher temperatures can favour the development of pathogens, while water scarcity (induced by droughts) affects water quality and hygiene habits, especially in arid and semi-arid areas, which could increase the burden of diseases (e.g. diarrhoea) (FAO, 2016), especially among the poor children. Indeed, the impacts of malnutrition caused by global warming might be

particularly severe among vulnerable groups such as children (Belesova et al., 2019; Sorgho et al., 2016) and women (Bryan et al., 2018). Also the dietary patterns of indigenous communities will be particularly affected (Smith et al., 2019; Wesche & Chan, 2010); referring to the Inuit in the Canadian Arctic, Wesche and Chan (2010) argue that "Changing access to, availability of, quality of, and ability to use traditional food resources has implications for quality of diet. Nutritional implications of lower traditional food use include likely reductions in iron, zinc, protein, vitamin D, and omega-3 fatty acids, among others" (p. 361). CC will also affect food utilisation in other ways such as reducing the nutrient content of staple crops and increasing the risk of food contamination (Lake et al., 2012; Medina et al., 2017). Indeed, CC will have notable impacts on food utilisation, including nutrition and food safety (Lake et al., 2012). Lake et al. (2012) highlight the relation between food access and food utilisation dimensions of FS in the context of changing climate and suggest that "Increasing food prices may lower the nutritional quality of dietary intakes, exacerbate obesity, and amplify health inequalities. Altered conditions for food production may result in […] altered use of pesticides and veterinary medicines, and affect the main transfer mechanisms through which contaminants move from the environment into food. All these have implications for food safety and the nutritional content of food" (p. 1520). Moreover, the literature points to some potential links between CC and diet-related non-communicable diseases (NCDs) (Savage et al., 2020). Other scholars analyse the linkage between CC and food utilisation the other way round and point out to the need to move towards dietary patterns that have lower GHG emissions to mitigate CC (Lake et al., 2012). Esham et al. (2018) point out that little attention is paid to the impacts of CC on access and utilization dimensions of FS and highlight that "achieving food security necessitates action beyond building climate resilient food production systems to a holistic approach that is able to ensure climate resilience of the entire food system while addressing nutritional concerns arising from impacts of climate change" (p. 1017). This implies expanding the narrow focus from the impacts of the changing climate on crop yield and productivity to include the impact on the nutritional value of crops (Leisner, 2020) and, consequently, diets.

Climate change and food system stability

Climate variability and the increasingly frequent and intensive extreme climate events will affect the stability of food availability, access and use. This will likely happen through changes in seasonality, fluctuations in ecosystem productivity, increased risks and reduced predictability of food supply (FAO, 2016). This will be a major problem especially for SIDS and landlocked countries, which are more vulnerable to both food supply and trade disruptions. Referring to the islands of the South Pacific, Barnett (2011) shows that "climate change will adversely affect food systems in the region, including the supply of food from agriculture and fisheries, the ability of countries to import food, systems for the distribution of food, and the ability of households to purchase and

utilize food" (p. 229). CC will affect all the elements of and activities within the food system (HLPE, 2014) with cascading effects and far-reaching implications in terms of FS at global, national and local levels (Myers et al., 2017; Schnitter & Berry, 2019). The short-term effects of temperature increase and rainfall variability on crop yields can have long-term effects on global food system stability. In this regard, Wheeler and von Braun (2013) put that "A robust and coherent global pattern is discernible of the impacts of climate change on crop productivity that could have consequences for food availability. The stability of whole food systems may be at risk under climate change because of short-term variability in supply" (p. 508). Climate variability would also increase price volatility (Baldos & Hertel, 2015; Takle et al., 2013). The recognition of the impacts of CC on food availability, access and utilisation and their stability over time implies the need of adopting a food system approach. In this respect, Keller et al. (2018) point out that "Efficient responses require an understanding of the full spectrum of potential climate impacts on food utilization, access and availability, as well as on the underlying natural, built and governance systems" (p. 742).

FOOD SECURITY AND CLIMATE CHANGE MITIGATION

The relation between CC and FS is dual but much more attention is paid in the literature to the impacts of CC on food security. In this respect, Islam and Wong (2017) argue that "it seems that research on climate change and food in/security has often been one-sided; with climate change being identified as the cause of food insecurity and not how the systems in place to ensure food security have exacerbated the issue of climate change". Agriculture is a main contributor to CC, through GHG emissions, and one of the most affected sectors by CC (FAO, 2016; HLPE, 2012). Agriculture, forestry and other land uses (AFOLU) account for about a fifth of GHG emissions worldwide (FAO, 2016). Indeed, Torquebiau (2017) suggests that "Agriculture is probably the most climate-dependent human activity and is both victim and responsible for climate change, while it can also be a solution to the climate change crisis". In this context, sustainable intensification is presented as a win-win strategy to combine FS and CC mitigation (Ayantunde et al., 2020; Descheemaeker et al., 2016; van Loon et al., 2019). However, referring to cereal cropping in SSA, van Loon et al. (2019) conclude that while "intensification scenarios are clearly superior to expansion scenarios in terms of climate change mitigation" (p. 3720), "Intensification will come, depending on the nutrient use efficiency achieved, with large increases in nutrient inputs and associated GHG emissions" (p. 3720). Many scholars highlighted the benefits of climate-smart agriculture (CSA) (Abegunde et al., 2019; García de Jalón et al., 2017) both for CC mitigation and adaptation. Loboguerrero et al. (2019) argue that "Climate-smart agriculture can help foster synergies between productivity, adaptation, and mitigation". In this context, FAO (2016) suggests that "Food and agriculture must be central to global efforts to adapt to climate change, through policies and actions that address vulnerabilities

and risks and promote agricultural systems that are resilient and sustainable […] Delaying the transformation of the agricultural sectors will force poorer countries to fight poverty, hunger and climate change at the same time".

CONCLUSIONS

This paper presents a comprehensive review on the multifaceted relationships between CC and food security. It shows an increasing academic interest in the nexus between CC and the different dimensions of food security. However, most of the articles deal with the effects of CC on food availability and agriculture (especially crop production) while analyses regarding the remaining dimensions of FS are rather scarce. The effects of CC on food security, that are expected to intensify over time, vary across countries and world regions. Indeed, effects are expected to be higher in developing countries whose economies are very dependent on the performance of rain-fed agriculture. Furthermore, the review shows that there is a dual relationship between climate change and food security. Both strategies for CC mitigation and adaptation have an impact on food security. The multifaceted and multidimensional relations between CC and FS call for adopting reflexive governance arrangements and integrated policies that maximise co-benefits while addressing trade-offs between climate and FS (e.g. agriculture) policies. This is of paramount importance to make sure that strategies for CC mitigation and/or adaptation do not jeopardize efforts for ending hunger and eradicating all forms of malnutrition in the framework of the implementation of the 2030 Agenda for Sustainable Development. In this respect, further efforts are needed in science, policy and practice to foster transition towards low emissions and climate resilient agriculture and food systems to achieve the long-term climate neutrality and food security objectives. Likewise, it is necessary to promote approaches that deliver mitigation, adaptation and development co-benefits, especially in the Global South.

ACKNOWLEDGEMENTS

This work was carried out within the project SUSTLIVES (SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems) of the DeSIRA initiative (Development Smart Innovation through Research in Agriculture) financed by the European Union.

REFERENCES

Abegunde V. O., Sibanda M., Obi A. (2019). The Dynamics of Climate Change Adaptation in Sub-Saharan Africa: A Review of Climate-Smart Agriculture among Small-Scale Farmers. *Climate*, *7*(11), 132. https://doi.org/10.3390/cli7110132

Adhikari U., Nejadhashemi A. P., Woznicki S. A. (2015). Climate change and eastern Africa: a review of impact on major crops. *Food and Energy Security*, *4*(2), 110–132.

Akinbile C. O., Akinlade G. M., Abolude A. T. (2015). Trend analysis in climatic variables and impacts on rice yield in Nigeria. *Journal of Water and Climate Change*, *6*(3), 534–543.

Akinseye F. M., Ajeigbe H. A., Traore P. C. S., Agele S. O., Zemadim B., Whitbread, A. (2020). Improving sorghum productivity under changing climatic conditions: A modelling approach. *Field Crops Research*, *246*, 107685. https://doi.org/10.1016/j.fcr.2019.107685

Ayantunde A. A., Oluwatosin B. O., Yameogo V., van Wijk M. (2020). Perceived benefits, constraints and determinants of sustainable intensification of mixed crop and livestock systems in the Sahelian zone of Burkina Faso. *International Journal of Agricultural Sustainability*, *18*(1), 84–98. https://doi.org/10.1080/14735903.2019.1698494

Baldos U. L. C. & Hertel T. W. (2015). The role of international trade in managing food security risks from climate change. *Food Security*, *7*(2), 275–290.

Bandara J. S. & Cai Y. (2014). The impact of climate change on food crop productivity, food prices and food security in South Asia. *Economic Analysis and Policy*, *44*(4), 451–465.

Barnett J. (2011). Dangerous climate change in the Pacific Islands: food production and food security. *Regional Environmental Change*, *11*(S1), 229–237.

Belesova K., Gornott C., Milner J., Sié A., Sauerborn R., Wilkinson P. (2019). Mortality impact of low annual crop yields in a subsistence farming population of Burkina Faso under the current and a 1.5 °C warmer climate in 2100. *Science of The Total Environment 691*, 538–548. https://doi.org/10.1016/j.scitotenv.2019.07.027

Bryan E., Bernier Q., Espinal M., Ringler C. (2018). Making climate change adaptation programmes in sub-Saharan Africa more gender responsive: insights from implementing organizations on the barriers and opportunities. *Climate and Development 10*(5), 417–431.

Butt T. A., McCarl B. A., Angerer J., Dyke P. T., Stuth J. W. (2005). The economic and food security implications of climate change in Mali. *Climatic Change*, *68*(3), 355–378.

Capone R., Bottalico F., Ottomano Palmisano G., El Bilali H., Dernini S. (2018). Food systems sustainability, food security and nutrition in the Mediterranean region: The contribution of the Mediterranean diet. In, Encyclopedia of Food Security and Sustainability, Volume 2. Elsevier, Amsterdam. pp. 176-180. https://doi.org/10.1016/B978-0-08-100596-5.21977-X

Capone R., El Bilali H., Debs Ph., Bottalico F. (2016). Relations between food and nutrition security, diets and food systems. *Agriculture and Forestry* 62(1): 49-58. DOI: 10.17707/AgricultForest.62.1.05.

Cadro, S., Uzunović, M., Cherni-Cadro, S., Zurovec, J. (2019). Changes in the water balance of Bosnia and Herzegovina as a result of climate change. *Agriculture and Forestry*, 65 (3): 19-33

Committee on World Food Security. (2012). *Coming to terms with terminology: Food security, Nutrition security, Food security and nutrition, Food and nutrition security*. Rome.

Cooper P. J. M., Dimes J., Rao K. P. C., Shapiro B., Shiferaw B., Twomlow S. (2008). Coping better with current climatic variability in the rain-fed farming systems of sub-Saharan Africa: An essential first step in adapting to future climate change? *Agriculture, Ecosystems & Environment*, *126*(1–2), 24–35. https://doi.org/10.1016/j.agee.2008.01.007

Čustović H., Đikić M., Ljuša M., Žurovec O. (2012). Effect of Climate Changes on Agriculture of the Western Balkan Countries and Adaptation Policies. *Agriculture & Forestry*, 58 (2): 127-141.

Davenport F., Funk C., Galu G. (2018). How will East African maize yields respond to climate change and can agricultural development mitigate this response? *Climatic Change*, *147*(3–4), 491–506. https://doi.org/10.1007/s10584-018-2149-7

Descheemaeker K., Oosting S. J., Homann-Kee Tui S., Masikati P., Falconnier G. N., Giller, K. E. (2016). Climate change adaptation and mitigation in smallholder crop–livestock systems in sub-Saharan Africa: a call for integrated impact assessments. *Regional Environmental Change*, *16*(8), 2331–2343.

Dey M. M., Gosh K., Valmonte-Santos R., Rosegrant M. W., Chen O. L. (2016). Economic impact of climate change and climate change adaptation strategies for fisheries sector in Solomon Islands: Implication for food security. *Marine Policy*, *67*, 171–178.

Diao X., Hazell P., Resnick D., Thurlow J. (2007). *The Role of Agriculture in Development: Implications for Sub-Saharan Africa*. Washington D.C.: International Food Policy Research Institute. https://doi.org/10.2499/9780896291614RR153

Ding Q., Chen X., Hilborn R., Chen Y. (2017). Vulnerability to impacts of climate change on marine fisheries and food security. *Marine Policy*, *83*, 55–61.

El Bilali H. (2019). Research on agro-food sustainability transitions: where are food security and nutrition? *Food Security* 11(3):559-577. DOI: https://doi.org/10.1007/s12571-019-00922-1

El Bilali H., Callenius C., Strassner C., Probst L. (2018). Food and nutrition security and sustainability transitions in food systems. *Food and Energy Security*; e00154. https://doi.org/10.1002/fes3.154

El Bilali H. (2018). Relation between innovation and sustainability in the agro-food system. *Italian Journal of Food Science* 30: 200-225. https://doi.org/10.14674/IJFS-1096

Ericksen P. J. (2008). Conceptualizing food systems for global environmental change research. *Global Environmental Change*, *18*(1), 234–245.

Esham M., Jacobs B., Rosairo H.S.R., Siddighi B.B. (2018). Climate change and food security: A Sri Lankan perspective. *Environment, Development and Sustainability 20*(3): 1017–1036. https://doi.org/10.1007/s10668-017-9945-5

FAO (2009). *Declaration of the World Food Summit on Food Security*. Rome.

FAO (2014). *Building a common vision for sustainable food and agriculture - Principles and Approaches*. Rome.

FAO (2016). *The State of Food and Agriculture: Climate Change, Agriculture and Food Security*. Rome.

FAO, IFAD, UNICEF, WFP & WHO (2019). *The State of Food Security and Nutrition in the World 2019 - Safeguarding against economic slowdowns and downturns*. Rome.

FAO, WFP & IFAD (2013). *The State of Food Insecurity in the World 2013: The Multiple Dimensions of Food Security*. Rome.

Freduah B., MacCarthy D., Adam M., Ly M., Ruane A., Timpong-Jones E., … Adiku S. (2019). Sensitivity of Maize Yield in Smallholder Systems to Climate Scenarios in Semi-Arid Regions of West Africa: Accounting for Variability in Farm Management Practices. *Agronomy*, *9*(10), 639. https://doi.org/10.3390/agronomy9100639

García de Jalón S., Iglesias A., Neumann M. B. (2018). Responses of sub-Saharan smallholders to climate change: Strategies and drivers of adaptation. *Environmental Science & Policy*, *90*, 38–45. https://doi.org/10.1016/j.envsci.2018.09.013

García de Jalón S., Silvestri S., Barnes A. P. (2017). The potential for adoption of climate smart agricultural practices in Sub-Saharan livestock systems. *Regional Environmental Change*, *17*(2), 399–410. https://doi.org/10.1007/s10113-016-1026-z

Godber O. F., Wall R. (2014). Livestock and food security: vulnerability to population growth and climate change. *Global Change Biology*, *20*(10), 3092–3102.

Gregory P., Ingram J.S., Brklacich M. (2005). Climate change and food security. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1463), 2139–2148.

Habiba U., Abedin M. A., Shaw R. (2016). Food Security, Climate Change Adaptation, and Disaster Risk. In J. Uitto & R. Shaw (Eds.), *Sustainable Development and Disaster Risk Reduction* (pp. 87–101). Tokyo: Springer.

Hasegawa T., Fujimori S., Havlík P., Valin H., Bodirsky B. L., Doelman J. C., … Witzke P. (2018). Risk of increased food insecurity under stringent global climate change mitigation policy. *Nature Climate Change*, *8*(8), 699–703.

HLPE (2012). *Food security and climate change. A report by the High Level Panel of Experts on food security and nutrition of the Committee on World Food Security*. Rome.

HLPE (2014). *Note on Critical and Emerging Issues for Food Security and Nutrition*. Rome.

Hussain A., Rasul G., Mahapatra B., Tuladhar S. (2016). Household food security in the face of climate change in the Hindu-Kush Himalayan region. *Food Security*, *8*(5), 921–937.

Intergovernmental Panel on Climate Change (2012). Sustainable Development and mitigation. In *Climate Change 2007* (pp. 691–744). Cambridge: Cambridge University Press.

Islam M. & Wong A. (2017). Climate Change and Food In/Security: A Critical Nexus. *Environments*, *4*(2), 38. https://doi.org/10.3390/environments4020038

Keller M., Zamudio A. N., Bizikova L., Sosa A. R., Gough A. M. (2018). Food security and climate change from a systems perspective: community case studies from Honduras. *Climate and Development*, *10*(8), 742–754.

Kiselev S., Romashkin R., Nelson G. C., Mason-D'Croz D., Palazzo A. (2013). Russia's Food Security and Climate Change: Looking into the Future. *Economics*, *7*(2013–39), 1. https://doi.org/10.5018/economics-ejournal.ja.2013-39

Lake I. R., Hooper L., Abdelhamid A., Bentham G., Boxall A. B. A., Draper A., … Waldron K. W. (2012). Climate Change and Food Security: Health Impacts in Developed Countries. *Environmental Health Perspectives*, *120*(11), 1520–1526.

Lam V., Cheung W., Swartz W., Sumaila U. (2012). Climate change impacts on fisheries in West Africa: implications for economic, food and nutritional security. *African Journal of Marine Science*, *34*(1), 103–117. https://doi.org/10.2989/1814232X.2012.673294

Lauria V., Das I., Hazra S., Cazcarro I., Arto I., Kay S., … Fernandes J. A. (2018). Importance of fisheries for food security across three climate change vulnerable deltas. *Science of The Total Environment*, *640–641*, 1566–1577.

Leisner C. P. (2020). Review: Climate change impacts on food security- focus on perennial cropping systems and nutritional value. *Plant Science*, *293*, 110412.

Loboguerrero A. M., Campbell B., Cooper P., Hansen J., Rosenstock T., Wollenberg E. (2019). Food and Earth Systems: Priorities for Climate Change Adaptation and Mitigation for Agriculture and Food Systems. *Sustainability*, *11*(5), 1372.

Mare F., Bahta Y. T., Van Niekerk W. (2018). The impact of drought on commercial livestock farmers in South Africa. *Development in Practice*, *28*(7), 884–898.

Medina A., Akbar A., Baazeem A., Rodriguez A., Magan, N. (2017). Climate change, food security and mycotoxins: Do we know enough? *Fungal Biology Reviews*, *31*(3), 143–154.

Moustache A. M. (2017). Adaptation to Impacts of Climate Change on the Food and Nutrition Security Status of a Small Island Developing State. In *Natural Resources Management* (pp. 919–944). Hersey (PA, USA): IGI Global.

Muchuru S. & Nhamo G. (2019). A review of climate change adaptation measures in the African crop sector. *Climate and Development*, *11*(10), 873–885.

Mugambiwa S. S. & Tirivangasi H. M. (2017). Climate change: A threat towards achieving 'Sustainable Development Goal number two' (end hunger, achieve food security and improved nutrition and promote sustainable agriculture) in South Africa. *Jàmbá: Journal of Disaster Risk Studies*, *9*(1). https://doi.org/10.4102/jamba.v9i1.350

Murray-Tortarolo G. N., Jaramillo V. J., Larsen J. (2018). Food security and climate change: the case of rainfed maize production in Mexico. *Agricultural and Forest Meteorology*, *253–254*, 124–131. https://doi.org/10.1016/j.agrformet.2018.02.011

Myers S. S., Smith M. R., Guth S., Golden C. D., Vaitla B., Mueller N. D., … Huybers P. (2017). Climate Change and Global Food Systems: Potential Impacts on Food Security and Undernutrition. *Annual Review of Public Health*, *38*(1), 259–277.

Naah J.-B. & Braun B. (2019). Local agro-pastoralists' perspectives on forage species diversity, habitat distributions, abundance trends and ecological drivers for sustainable livestock production in West Africa. *Scientific Reports*, *9*(1), 1707.

Poudel S., Funakawa S., Shinjo H. (2017). Household Perceptions about the Impacts of Climate Change on Food Security in the Mountainous Region of Nepal. *Sustainability*, *9*(4), 641.

Qureshi M., Hanjra M. A., Ward J. (2013). Impact of water scarcity in Australia on global food security in an era of climate change. *Food Policy*, *38*, 136–145.

Ramasamy S. (2010). World Food Security: The Challenges of Climate Change and Bioenergy. In R. Lal, M. Sivakumar, S. Faiz, A. Rahman, & K. Islam (Eds.), *Climate Change and Food Security in South Asia* (pp. 183–213). Dordrecht: Springer.

Roudier P., Sultan B., Quirion P., Berg A. (2011). The impact of future climate change on West African crop yields: What does the recent literature say? *Global Environmental Change*, *21*(3), 1073–1083. https://doi.org/10.1016/j.gloenvcha.2011.04.007

Rudi L.-M., Azadi H., Witlox F. (2012). Reconcilability of Socio-Economic Development and Environmental Conservation in Sub-Saharan Africa. *Global and Planetary Change*, *86–87*, 1–10.

Savage A., McIver L., Schubert L. (2020). Review: the nexus of climate change, food and nutrition security and diet-related non-communicable diseases in Pacific Island Countries and Territories. *Climate and Development*, *12*(2), 120–133.

Schnitter R. & Berry P. (2019). The Climate Change, Food Security and Human Health Nexus in Canada: A Framework to Protect Population Health. *International Journal of Environmental Research and Public Health*, *16*(14), 2531.

Simunic, I., Likso, T., Miseckaite, O., Orlović-Leko, P., Ciglenečki, I., Spalević, V. (2019). Climate changes and soil water regime. *Agriculture and Forestry*, 65(3): 05-18. DOI: 10.17707/AgricultForest.65.3.01

Smith E., Ahmed S., Dupuis V., Running Crane M., Eggers M., Pierre M., … Byker Shanks C. (2019). Contribution of Wild Foods to Diet, Food Security, and Cultural Values Amidst Climate Change. *Journal of Agri., Food Syst. and Com. Dev.*, *9*, 1–24.

Solaymani, S. (2018). Impacts of climate change on food security and agriculture sector in Malaysia. *Environment, Development and Sustainability*, *20*(4), 1575–1596.

Sorgho R., Franke J., Simboro S., Phalkey R., Saeurborn R. (2016). NUTRItion and CLIMate (NUTRICLIM): investigating the relationship between climate variables and childhood malnutrition through agriculture, an exploratory study in Burkina Faso. *Public Health Reviews*, *37*(1), 16. https://doi.org/10.1186/s40985-016-0031-6

Steffen W., Richardson K., Rockstrom J., Cornell S. E., Fetzer I., Bennett E. M., … Sorlin S. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science*, *347*(6223), 1259855–1259855. https://doi.org/10.1126/science.1259855

Sultan B., Defrance D., Iizumi T. (2019). Evidence of crop production losses in West Africa due to historical global warming in two crop models. *Scientific Reports*, *9*(1), 12834.

Sultan B. & Gaetani M. (2016). Agriculture in West Africa in the Twenty-First Century: Climate Change and Impacts Scenarios, and Potential for Adaptation. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.01262

Takle E. S., Gustafson D., Beachy R., Nelson G. C., Mason-D'Croz D., Palazzo A. (2013). US Food Security and Climate Change: Agricultural Futures. *Economics 7*(2013–34), 1. https://doi.org/10.5018/economics-ejournal.ja.2013-34

Tamako N. & Thamaga-Chitja J. M. (2017). Does social capital play a role in climate change adaptation among smallholder farmers for improving food security and livelihoods? *Food and Nutrition Challenges in Southern Africa*, 16–27.

Torquebiau E. (2017). Le changement climatique, un défi pour la recherche : l'exemple de l'initiative « 4 ‰ ». *OCL*, *24*(1), 108. https://doi.org/10.1051/ocl/2016054

Trnka M., Feng S., Semenov M. A., Olesen J. E., Kersebaum K. C., Rötter R. P., … Büntgen U. (2019). Mitigation efforts will not fully alleviate the increase in water scarcity occurrence probability in wheat-producing areas. *Science Advances*, *5*(9), e2406.

United Nations (2015a). *The Millennium Development Goals Report 2015*. New York.

United Nations (2015b). *Transforming Our World: The 2030 Agenda for Sustainable Development. Resolution adopted by the General Assembly on 25 September 2015*. New York.

United Nations System High Level Task Force on Global Food Security (2011). *Food and Nutrition Security: Comprehensive Framework for Action. Summary of the Updated Comprehensive Framework for Action (UCFA)*. Rome.

van Loon M. P., Hijbeek R., Berge H. F. M., De Sy V., Broeke G. A., Solomon D., Ittersum M. K. (2019). Impacts of intensifying or expanding cereal cropping in sub-Saharan Africa on greenhouse gas emissions and food security. *Global Change Biology 25*(11), 3720–3730. https://doi.org/10.1111/gcb.14783

van Oort P. A. J. & Zwart S. J. (2018). Impacts of climate change on rice production in Africa and causes of simulated yield changes. *Global Change Biology*, *24*(3), 1029–1045.

Waha K., Müller C., Rolinski S. (2013). Separate and combined effects of temperature and precipitation change on maize yields in sub-Saharan Africa for mid- to late-21st century. *Global and Planetary Change*, *106*, 1–12.

Webersik C. & Wilson C. (2009). Achieving environmental sustainability and growth in Africa: the role of science, technology and innovation. *Sustainable Development*, *17*(6), 400–413. https://doi.org/10.1002/sd.411

Wesche S. D. & Chan H. M. (2010). Adapting to the Impacts of Climate Change on Food Security among Inuit in the Western Canadian Arctic. *EcoHealth*, *7*(3), 361–373.

Wheeler T. & von Braun J. (2013). Climate Change Impacts on Global Food Security. *Science*, *341*(6145), 508–513. https://doi.org/10.1126/science.1239402

Williams P. A., Crespo O., Abu M., Simpson N. P. (2018). A systematic review of how vulnerability of smallholder agricultural systems to changing climate is assessed in Africa. *Environmental Research Letters*, *13*(10), 103004.

Wood S. A., Jina A. S., Jain M., Kristjanson P., DeFries R. S. (2014). Smallholder farmer cropping decisions related to climate variability across multiple regions. *Global Environmental Change*, *25*, 163–172. https://doi.org/10.1016/j.gloenvcha.2013.12.011

Zougmoré R. B., Partey S. T., Ouédraogo M., Torquebiau E., Campbell B. M. (2018). Facing climate variability in sub-Saharan Africa: analysis of climate-smart agriculture opportunities to manage climate-related risks. *Cahiers Agricultures*, *27*(3), 34001.

Bašić, M., Beganović, L., Huremović, J., Žero, S. (2020): Assessment of metals and their estimated daily intakes in various nuts. Agriculture and Forestry, 66 (3): 211-219.

DOI: 10.17707/AgricultForest.66.3.17

Midheta BAŠIĆ, Lejla BEGANOVIĆ, Jasna HUREMOVIĆ[1], Sabina ŽERO

ASSESSMENT OF METALS AND THEIR ESTIMATED DAILY INTAKES IN VARIOUS NUTS

SUMMARY

Nuts have received increased public attention in recent years as important sources of essential elements. Information on the levels of elements in edible nuts is useful to consumers. Content of metals was analysed in various types of nuts from Sarajevo markets, Bosnia and Herzegovina. The samples included almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios. Metals cadmium, chromium, iron, manganese, lead and zinc (Cd, Cr, Fe, Mn, Pb and Zn) were determined by flame atomic absorption spectrometry (FAAS). Moreover, estimated daily intake of all analysed metals and carcinogenic risk over a lifetime exposure to Pb has been calculated.

The metal levels in nuts ranged as follows: 1.53-6.95 mgCd/kg, 1.97-7.92 mgCr/kg, 32.03-97.70 mgFe/kg, 6.48-30.58 mgMn/kg, 0.42-1.38 mgPb/kg, 31.30-50.23 mgZn/kg. By concentrations in nuts, in most cases, the metals were arranged as the following diminishing series: Fe > Pb > Zn >Mn > Cr > Cd. Estimated daily intakes for Cd, Cr, Fe, Mn, Pb and Zn were calculated and varied from 0.0002 mg/kg/day for Pb to 0.0489 mg/kg/day for Fe. Carcinogenic risk for Pb was lower than 10^{-6} indicating the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range. The results provide important information on the nutritional values of nut samples at the Sarajevo market. In most cases, the content of metal was in accordance with the contents given in the previously published papers.

Keywords: Nuts, Metals, Contamination, Daily Intake, Carcinogenic Risk.

INTRODUCTION

Food crops like fruits, vegetables and nuts cultivated in contaminated lands can accumulate toxic heavy metals. Human exposure to toxic metals goes through ingestion of contaminated food and water and by inhalation of air pollutants or contaminated soil particles (Davarynejad et al. 2013; Onyedikachi et al. 2018). In the past years, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of contaminated foodstuffs by

[1]Jasna Huremović (corresponding author: jasnahuremovic@yahoo.com), Midheta Bašić, Lejla Beganović, Sabina Žero, University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, BOSNIA AND HERZEGOVINA.

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:15/07/2020 *Accepted:16/09/2020*

heavy metals and pesticides or toxins (D'Mello 2003). Nuts are widely used in the food industry such as baking, confectionery, candy and chocolate products, ice-cream and dairy (Özkutlu et al. 2013). Nuts have received increased public attention in recent years as important sources of essential elements, and information on the levels of elements in edible nuts is useful to consumers (Yin et al. 2015). Bioactive compounds, such as essential fatty acids, essential amino acids, antioxidants, essential mineral and vitamins are present in high concentrations in nuts. Thus, health benefits which we have by consuming nuts are blood pressure and body weight control, reduction of blood cholesterol and triglycerides, as well as prevention of coronary heart disease (Moreda-Piñeiro et al. 2016). Determination of some elements in nuts is not only necessary in evaluating the dietary intake of the essential elements, but also useful in detecting heavy metal contamination in food (Cabrera et al. 2003; Chung et al. 2013; Yin et al. 2015).

Nuts are rich in micronutrients such as niacin and folic acid, vitamins (E and B6) and minerals (Ca, Cr, Mg, Mn, Cu, Fe, Zn, Se, P and K) (Kafaoglu et al. 2016). Fe, Cu, Cr and Zn are essential micronutrients for human health. They play an important role in human metabolism, and interest in essential micronutrients is increasing together with reports of relationships between trace element status and oxidative diseases (Cabrera et al. 2003). Some heavy metals, if present even in very low concentrations in food products, can cause human health problems. Information about the dietary intake of such metals is very important to assess risks to consumers (Lanre-Iyanda and Adekunle 2012). Also, the determination of trace elements and minerals in foodstuffs is an important part of toxicological and nutritional analyses (Cabrera et al. 2003). Biotoxic effects of heavy metals depend upon the oxidation states and concentrations of heavy metals, mode of deposition and kind of sources (Bukvić et al. 2013).

The aim of this study was to analyse the metals cadmium, chromium, iron, manganese, lead and zinc (Cd, Cr, Fe, Mn, Pb, Zn) concentrations in different types of nuts marketed in Sarajevo: almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios. Metals were analysed by flame atomic absorption spectrometry (FAAS). In addition, estimated daily intakes of metals from nuts samples and estimated carcinogenic risk over a lifetime exposure to Pb were also calculated.

MATERIAL AND METHODS

Sample collection and analysis

Nuts samples were collected from different Sarajevo (Bosnia and Herzegovina) markets. Samples of nuts were collected randomly in sufficient quantities to provide a representative samples. Samples were cut to small pieces and dried at 105ºC, then powdered and stored in plastic bags for metals analysis. Metals in nuts samples were extracted following the acid digestion procedure: a total of about 1 g (± 0.1 mg) of dry nuts was weight directly in Teflon digestion vessels. Following the addition of 30 mL 65% (v/v) HNO_3, after the evaporation

of nitrogen oxides, the vessel was sealed and allowed to react at 50ºC for 12 h. The digest was transferred to a volumetric flask and filled up with Milli-Q water to 50 mL. Metals content of commonly consumed nuts were determined by FAAS using an atomic absorption spectrometer model AA240FS, Varian (Australia). All samples and blanks were prepared in triplicate and measurement for each replicate was repeated thrice. All the chemicals and reagents were of analytical grade and were purchased from Merck (Germany). Standard solutions of metals were also provided by Merck (Germany). Milli-Q water was used throughout the whole experiment. All of the volumetric glassware used were soaked in 10% (v/v) HNO_3 overnight and rinsed with Milli-Q water before use. The sample analyses were performed in triplicate and the standard deviation was calculated. The method detection limits (LOD) were calculated as three times the standard deviation of the blank signal. The LOD values were: Cr (0.006 mg/L), Mn (0.002 mg/L), Fe (0.006 mg/L), Pb (0.01 mg/L), Ni (0.01 mg/L), Cd (0.002 mg/L), Zn (0.001 mg/L). Spiked samples were used to test the methods at varying concentrations of analyte and the percent recovery was calculated. The acceptable recoveries of known additions ranged between 89-108%. Metal concentrations are expressed as the mean value (mg/kg) ± standard deviation (SD) of three subsamples collected from the same source.

Estimated daily intake of metals (EDI)

A recommended dietary intake (RDI), sometimes referred to as recommended daily intake, is the average daily intake level of a particular nutrient that is likely to meet the nutrient requirements of 97-98% of healthy individuals in a particular life stage or gender group. Estimated daily intake (mg/kg/day) is calculated based on the assumptions that: a) body weight is 60 kg and b) daily intake of nuts is 30 g. Estimated daily intake were calculated using (mg/kg/day) = metal concentrations in nuts × 30/1000/60 (Ozbas et al. 2013).

Carcinogenic risk (CR)

Carcinogenic risk (CR) shows the probability of developing cancer over a lifetime due to exposure to a potential carcinogen for an individual. The United States Environmental Protection Agency (USEPA 2010) provided a cancer slope factor (CSF) for the calculation of the cancer risk over a lifetime exposure to Pb. The estimation of the cancer risk is calculated using the Equation 1 given by USEPA: $CR = CSF \times ED$ /1/

where, CSF is the carcinogenic slope factor of 0.0085 (mg/kg/day) for Pb set by USEPA (2010). Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000).

RESULTS AND DISCUSSION

Nuts are known to be a concentrated food for major elements and they are an interesting food for trace elements for the human diet. A small number of published data are available on the metal contamination in nuts on different market sites.

Moisture content in nuts. The knowledge of real moisture distribution of nut and their components is an essential requirement for obtaining desired high-quality products and designing of efficient processing operations (Khir et al. 2013). Like many other products, nuts must have moisture content where they are dry enough to meet customer quality specifications but not so dry that they break during shipment. For example, moisture content in walnuts is a sensitive parameter. Target moisture content is around 4.6% (Nielsen 2010). The Food and Drug Administration regulations for tree nuts define a safe moisture level (moisture content that does not support fungal growth) which is typically 8% on a wet basis (Khir et al. 2013). Moisture content (%) in nuts is presented in Table 1. Moisture content in all analysed samples ranged from 1.62% (Macadamia) to 4.26% (Indian walnuts). Thus, the obtained values were common values for the moisture content in such nutritional products.

Table1. Moisture content (%) in nuts

Sample	Moisture content (%)
Almond	4.06
Walnuts	3.78
Brazil walnuts	3.02
Indian walnuts	4.26
Macadamia	1.62
Hazelnuts	3.77
Pistachios	3.47

Metal content in nuts. All metal concentrations were determined on a dry weight basis. Metal contents in different types of nuts marketed in Sarajevo are presented in Table 2.

Table 2. Metal concentration (mg/kg ± standard deviation) in different types of nuts (n = 3)

Metal concentration (mg/kg) ± standard deviation							
Metal	Sample						
	Brazil walnuts	Pistachios	Indian walnuts	Almond	Walnuts	Hazelnuts	Macadamia
Cd	1.53±0.36	2.64±0.31	2.91±0.03	3.35±0.08	3.85±0.19	6.95±2.78	4.81±0.15
Cr	6.46±4.15	7.92±0.82	5.58±0.85	2.90±1.51	7.17±1.16	3.34±1.09	1.97±0.30
Fe	40.96±28.37	97.7±21.90	69.5±28.11	32.03±15.96	70.16±17.5	19.39±3.39	54.50±7.78
Mn	6.48±4.37	7.37±1.67	14.88±1.10	10.88±2.10	24.36±1.07	11.02±5.21	30.56±2.40
Pb	0.61±0.26	0.42±0.04	0.53±0.01	0.70±0.02	0.64±0.03	1.38±0.20	0.84±0.08
Zn	33.08±17.12	36.43±0.60	50.23±3.60	37.1±16.00	41.6±2.52	49.91±19.70	31.3±4.04

By concentrations in nuts, in most cases, the metals were arranged as the following diminishing series: Fe > Pb > Zn > Mn > Cr > Cd. Published data on the content of metals in nuts around the world are presented in Table 3.

Table 3. Literature values of metal content in nuts from various parts of the world

Samples	Cd (mg/kg)	Cr (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Brazil walnut	n.a.	n.a.	21.29±1.36 [6]	11.98±0.16 [6]	n.a.	32.06±1.5 [6]
Pistachio	0.07±0.01 [3] 0.45±0.23 [5] n.d. [4]	0.60 - 1.86 [1] 0.15±0.08 [3] 0.37 - 0.41 [4]	24.92±3.87 [6] 88.67±6.49 [5] 70.0 - 75.6 [4]	6.0±1.2 [3] 9.11±0.23 [6]	<LOD [3] 11.62±1.42 [5] 0.15 - 0.25 [4]	15±3.3 [3] 16.81±0.65 [6] 67.24±30.25 [5] 30.2 - 36.7 [4]
Almond	0.04±0.01 [3] 0.24 [7] 0.48±0.24 [5] n.d.-0.012 [4]	0.31±0.038 [3] 0.39-0.49 [4]	20.57±1.92 [6] 62.10±5.86 [5] 40.3-48.7 [4]	14±4.5 [3] 21.13±0.94 [6]	<LOD [3] 1.02 [7] 2.81±0.56 [5] 0.26-0.37 [4]	24±4.0 [3] 27.31±0.98 [6] 19.16±16.21 [5] 27.9-50.0 [4]
Walnut	0.02±0.00 [3] 24.23±1.62 [6] 0.49±0.32 [5] n.d.-0.009 [4]	<LOD [3] 0.001 [8] 0.30-0.38 [4]	22.08±1.01 [6] 57.42±22.24 [5] 0.064 [8] 20.0-24.4 [4]	10±0.12 [3] 0.012 [8]	<LOD [3] 3.43±0.22 [5] 0.20-0.26 [4]	20±1.1 [3] 23.25±0.50 [6] 15.21±12.86 [5] 25.6-39.7 [4]
Hazelnut	0.24±0.00 [3] 0.01-0.03 [2]	0.22±0.02 [3] 0.02-0.05 [2]	25.95±1.02 [6] 35.1-49.4 [2]	33±9.8 [3] 53.47±1.73 [6] 41.1-116.81 [2]	0.17±0.14 [3] 0.02-0.07 [2]	15±0.82 [3] 16.02±0.15 [6] 23.5-35.5 [2]
Macadamia	0.05±0.00 [3] 0.46±0.23 [5]	0.090±0.060 [3]	57.68±8.23 [5]	11.6±0.17 [3]	0.18±0.02 [3] <LOD [5]	8.6±1.5 [3] 9.01±7.64 [5]
Indian Walnut	n.a.					

[1]- (Davarynejad *et al.* 2013); [2]- (Özkutlu *et al.* 2013); [3]- (Yin *et al.* 2015); [4]- (Cabrera *et al.* 2003); [5]- (Chung *et al.* 2013); [6]- (Kafaoglu *et al.* 2016); [7]- (Sattar *et al.* 1989) [8]- (Edem *et al.* 2008); n.a. - not available

Cadmium has no known biological functions (ATSDR 2008). It interferes with some essential function of Zn, thereby inhibiting nutrient utilization and enzyme reactions. Cadmium catalyses oxidation reactions and generates free-radical damage of tissue (Lanre-Iyanda and Adekunle 2012). Concentrations of Cd in nuts samples were found between 1.53 (Brazil walnuts) and 6.95 mg/kg (hazelnuts). If comparing the results from this study with results from other countries shows that the Cd content was similar or slightly higher in this study. According to the Ordinance on Maximum Permissible Quantities for Certain Contaminants in Food (Official Gazette of BiH 2014) the maximum permissible amount of Cd for example in wheat and rice (there is no defined maximum permissible amount in nuts) is 0.20 mg/kg of wet weight.

Chromium concentrations in human tissues decline with age, except for the lungs in which Cr accumulates. Parity, juvenile diabetes, and coronary artery disease are associated with low Cr content rations in serum or hair (Kafaoglu et al. 2016). Significant variation in Cr concentration was found among the nut samples. Values ranged from 1.97 mg/kg in Macadamia to 7.92 mg/kg in pistachios and were slightly higher in regard to previous published results given in Table 3. The results have shown that pistachio is an excellent source of Cr.

Consuming 3.5 g of pistachio per day covers the adequate daily intake of an adult (woman). The intake of too much of Cr can cause harmful health effects for instance hepatitis, ulcers, gastritis and lung cancer (Davarynejad et al. 2013).

Iron as essential metal plays a vital role in human physiology mainly in the formation of hemoglobin, electron and oxygen transport in human body (Nejabat et al. 2017). Fe concentrations ranged from 19.39 (hazelnut) to 97.70 mg/kg in pistachios. According to results of Fe content in nuts and comparing these results with recommended daily Fe intake, it seems that consumption of around 80 g/day of pistachios can provide the total Fe need in the human body of men. Samples were arranged as the following diminishing series by Fe concentrations: pistachios > walnuts > Indian walnuts > Macadamia > Brazil walnuts > almond > hazelnuts.

Manganese serves as a co-factor for many metabolic and physiological functions (Yin et al. 2015). Concentrations of Mn in nuts samples were found between 6.48 mg/kg in Brazil walnuts and 30.56 mg/kg in Macadamia. Comparing the results from this study with adequate daily Mn intake value, it seems that consumption of 15 g/day of Macadamia or walnuts can provide the total Mn need of woman.

Toxic compounds find their way into food during manufacture, transportation or storage, these include largely heavy metals. Lead presence in food may be influenced by industries, heavily travelled highways, and urban communities in combination with Pb supplied through irrigation water, pesticides and fertilizers (Cabrera et al. 2003). Pb is one of the representative elements whose levels in the environment represent a reliable index of environmental pollution (Yin et al. 2015). Pb levels in this study ranged from 0.42 (pistachios) to 1.38 mg/kg (hazelnuts) and were similar to the values from previously published data (Table 3). The carcinogenic risk over a lifetime exposure to Pb was calculated and presented in Table 4. Generally, the values of CR lower than 10^{-6} are considered as negligible, above 10^{-4} are considered to be unacceptable and lying in between 10^{-6} and 10^{-4} are considered as acceptable range (Atique et al. 2017). In this study, CR for Pb was lower than 10^{-6} indicating the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range.

The net delivery of Zn to an organism is a function of its bioavailability and of the total amount of this element in foods. Zn levels determined in nuts ranged from 31.33 mg/kg in Macadamia to 50.23 mg/kg in Indian walnuts. According to results of Zn content in nuts and comparing these results with recommended daily Zn intake, consumption of about 140 g of Indian walnuts can provide the total Zn need in the human body of woman. By comparing the obtained results with results from other countries it is shown that the Zn content was very similar or higher in the case of Macadamia walnuts.

Estimated daily intakes of metals from nuts. The estimated daily intake of metals (Cd, Cr, Fe, Mn, Pb and Zn) depended on both the metal concentration level and the amount of consumption of nuts. Results of estimated daily intakes for all metals from nuts samples are presented in Table 5.

Table 4. Estimated carcinogenic risk over a lifetime exposure to Pb

Pb/ Sample	Carcinogenic risk (CR)
Brazil Walnut	2.59E-06
Pistachio	1.79E-06
Indian Walnut	2.25E-06
Almond	2.98E-06
Walnut	2.72E-06
Hazelnut	5.87E-06
Macadamia	3.57E-06

Table 5. Estimated daily intakes of metals from nuts

	Estimated daily intakes (mg/kg/day)					
Metal/ Sample	Cd	Cr	Fe	Mn	Pb	Zn
Brazil Walnut	0.0008	0.0032	0.0205	0.0032	0.0003	0.0165
Pistachio	0.0013	0.0040	0.0489	0.0037	0.0002	0.0182
Indian Walnut	0.0015	0.0028	0.0348	0.0074	0.0003	0.0251
Almond	0.0017	0.0015	0.0160	0.0054	0.0004	0.0186
Walnut	0.0019	0.0036	0.0351	0.0122	0.0003	0.0208
Hazelnut	0.0035	0.0017	0.0097	0.0055	0.0007	0.0250
Macadamia	0.0024	0.0010	0.0273	0.0153	0.0004	0.0157

It was shown that the calculated EDI values for daily average consumption (30 g) ranged from 0.0002 mg/kg/day for Pb in pistachio to 0.0489 mg/kg/day for Fe in pistachio. The recommended daily intake of Fe for adults ranges from 8 mg/day for men and 18 mg/day for women, for Zn from 9.4 mg/day for men and 6.8 mg/day for woman, adequate intake of Cr is 35 μg/day for men and 25 μg/day for woman and for Mn is 2.3 mg/day for men and 1.8 mg/day for woman (Official Gazette of BiH 2014).

CONCLUSIONS

Food contamination with heavy metals may come from food processing and packaging, therefore, monitoring of nuts from markets is very important. For this study nut samples were collected from markets in Sarajevo. In spite of the known major mineral composition of K, P, Mn, Ca, Mg, Fe and other in nuts, there is scarce literature on trace and heavy element contents like Cd, Cr, Pb and Zn. Total elemental concentrations of Cd, Cr, Fe, Mn, Pb and Zn in nuts have been determined in several types of nut, including almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios.

The results provide important information on the nutritional values of nut samples at the Sarajevo market. In most cases, the content of metal was in accordance with the contents given in the previously published papers. The

calculated carcinogenic risk for Pb is indicating that the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range.

REFERENCES

Atique Ullah, A.K.M., Maksud, M.A., Khan, S.R., Lutfa, L.N., Quraishi, S.B. (2017): Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. *Toxicol Rep.*, 4: 574–579. https://doi.org/10.1016/j.toxrep.2017.10.002

ATSDR (2008): C*ase Studies in Environmental Medicine (CSEM), Cadmium Toxicity*. Agency for Toxic Substances and Disease Registry (availabele at https://www.atsdr.cdc.gov/csem/cadmium/docs/cadmium.pdf)

Bukvić, E., Huremović, J., Memić, M., Gojak-Salimović, S. (2013): Heavy metals in fruits and vegetables from markets in Sarajevo, Bosnia and Herzegovina. *Technol Acta.,* 6(2): 29-36.

Cabrera, C., Loris, F., Gimenez, R., Olalla, M., López, M.C. (2003): Mineral content in legumes and nuts: contribution to the Spanish dietary intake. *Sci Total Environ.,* 308: 1–14. https://doi.org/10.1016/S0048-9697(02)0061-3

Chung, K.H., Shin, K.O., Hwang, H.J., Choi, K.S. (2013): Chemical composition of nuts and seeds sold in Korea. *Nutr Res Pract.,* 7(2): 82–88. https://doi.org/10.4162/nrp.2013.7.2.82

D'Mello, J.P.F. (2003): *Food safety: contaminants and toxins*. Wallingford, Oxon, UK, Cambridge, MA, CABI Publishing.

Davarynejad, G., Zarei, M., Nagy, P.T. (2013): Identification and quantification of heavy metals concentrations in pistacia. *Not Sci Biol.,* 5(4): 438-444. https://doi.org/10.15835/nsb549115

Edem, C.A., Dosumu, M.I., Bassey, F.I. (2008): Determination of proximate composition, ascorbic acid and heavy metal content of African walnut (Tetra carpidium conophorum). *Afr J Environ Pollut Health.,* 6(1): 59-60. http://dx.doi.org/10.3923/pjn.2009.225.226

Kafaoglu, B., Fisher, A., Hill, S., Karaa, D.J. (2016): Determination and Evaluation of Metal Bioaccessibility in Some Nuts and Seeds by in Vitro Gastro-Intestinal Method. *J Food Composit Anal.,* 45: 58-65. https://doi.org/10.1016/j.jfca.2015.09.011

Khir, R., Pan, Z., Atungulu, G.G., Thompson, J.F., Shao, D. (2013): Size and Moisture Distribution Characteristics of Walnuts and Their Components. *Food Bioproc Tech.,* 6(3): 771–782. https://doi.org/10.1007/s11947-011-0717-1

Lanre-Iyanda, T.Y. & Adekunle, I.M. (2012): Assessment of heavy metals and their estimated daily intakes from two commonly consumed foods (kulikuli and robo) found in Nigeria. *Afr J Food Agric Nutr Dev.,* 12(3): 6156.

Moreda-Piñeiro, J., Herbello-Hermelo, P., Domínguez-González, R., Bermejo-Barrera, P., Moreda-Piñeiro, A. (2016): Bioavailability assessment of essential and toxic metals in edible nuts and seeds. *Food Chem.,* 205:146-154. https://doi.org/10.1016/j.foodchem.2016.03.006

Nejabat, M., Kahe, H., Shirani, K., Ghorbannejad, P., Hadizadeh, F., Karimi, G. (2017): Health risk assessment of heavy metals via dietary intake of wheat in Golestan Province, Iran. *Hum Ecol Risk Assess.*, 23(5): 1193-1201. https://doi.org/10.1080/10807039.2017.1309265

Nielsen, S.S. (2010): *Food analysis*, 4th edn., West Lafayette, IN, USA, Purdue University.

Official Gazette of BiH (2014): *Ordinance on Maximum Permissible Quantities for Certain Contaminants in Food*, No. 68/14 of the Food Act (available at http://www.sluzbenilist.ba/page/akt/zUp8Jgztz5k76kjn45hB4GvE=)

Onyedikachi, U.B., Belonwy, DC, Wegwu, MO. (2018): Human health risk assessment of heavy metals in soils and commonly consumed food crops from quarry sites located at Isiagwu, Ebonyi State. *Ovidius Univ Ann Chem.*, 29(1): 8-24. https://doi.org/10.2478/auoc-2018-0002

Ozbas, E.E., Okten, H.E., Ozcan, H.K., Demir, G. (2013): Selected Essential and Non-Essential Elements Content of Turkish Hazelnuts. *Environ Pollut.*, 2(1): 81-87. http://dx.doi.org/10.5539/ep.v2n1p81

Özkutlu, F., Doğru, Y.Z., Özenç, N., Yazici, G., Turan, M., Akçay, F. (2013): The importance of Turkish hazelnut trace and heavy metal contents for human nutrition. *S Afr J Plant Soil.*, 1(3): 37-44.

Sattar, A., Wahid, M., Durrani, S.K. (1989): Concentration of selected heavy metals in spices, dry fruits and plant nuts. *Plant Food Hum Nutr.*, 39(3): 279-286. https://doi.org/10.1007/BF01091938

USEPA (2010): *Risk-based Concentration Table.* United States Environmental Protection Agency (available at https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables)

Yin, L.L., Qing, T., Zhang, S.X,. Yin, K.X., Qin, J.I.Q. (2015): Determination of Trace Elements in Edible Nuts in the Beijing Market by ICP-MS. *Biomed Environ Sci.*, 28(6): 449-454. https://doi.org/10.3967/bes2015.063

Maličević, Z., Lakić, Ž., Jugović, M., Mitrović, D. (2020): The significance of transversal distribution during the application of plant protection by boom sprayer. Agriculture and Forestry, 66 (3): 221-230.
DOI: 10.17707/AgricultForest.66.3.18

Zoran MALIČEVIĆ [1], Željko LAKIĆ [2], Milan JUGOVIĆ [3], Dragoljub MITROVIĆ [4]

THE SIGNIFICANCE OF TRANSVERSAL DISTRIBUTION DURING THE APPLICATION OF PLANT PROTECTION BY BOOM SPRAYER

SUMMARY

The capacity of nozzles changes during the exploitation, i.e. it produces deviation from normal values. The consumption has an effect on the increase of the capacity of the nozzle, or the fraying and abrasion of the cartridge itself, which has a consequence of increased flow of the substance. On the other hand, decrease of the capacity occurs as a consequence of impurities which come with water which is used as a carrier of substances.

The aim of the study is to point to the significance of regular control of the nozzle capacity, since it affect on the quality of the application, all of which affect the deviation from the mean value of the transverse distribution.

Measured deviations of the nozzles according to the positions had significant deviations (5- decrease 83,33%, or 13- increase of the capacity for 61,11%), and the consumption of the substance according to the surface (ha) fit perfectly. During designing and monitoring of the treatment standard, most of the producers monitor consumption (l/ha), and this paper points to frequent occurrences in practice (deviations) that can significantly affect the quality of the application, as shown by water-sensitive papers. The coverage of water-sensitive papers ranged from 5.60 to 57.40%.

Keywords: nozzles, transversal distribution, boom sprayer, controlled application.

INTRODUCTION

In practice, it is often the case that manufacturers adjust the operating parameters (device capacity, operating speed) to achieve the desired norm in terms of consumption of the agent according to the treated area. Quality treatment, i.e. good transverse distribution implies uniform spray capacity as well

[1]Zoran Maličević (corresponding author: zoran.malicevic@agro.fabl.org), Faculty of Agriculture, University of Banja Luka, Republic of Srpska, BOSNIA AND HERZEGOVINA.
[2]Željko Lakić, Institute for Agriculture Banja Luka, Republic of Srpska, BOSNIA AND HERZEGOVINA.
[3]Milan Jugović, University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, BOSNIA AND HERZEGOVINA.
[4]Dragoljub Mitrović, Biotechnical Faculty, University of Montenegro, MONTENEGRO.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Recieved:19/06/2020 *Accepted:20/09/2020*

as good jet overlap. Change of the stated parameters occurs due to deviations from the nominal values of the capacity of individual sprayers on grains sprayers. Due to the deviation from the nominal values of the capacity, the quality application is disturbed through the uneven transverse distribution.

The mean value of the transverse distribution represents the mean volume value of the distributed liquid per treated area. A greater or lesser deviation from this mean represents the coefficient of variation. The coefficient of variation represents the numerical value of the distribution of fluid over the surface and allows the comparison of different results (Visacki, 2014).

The result of the treatment with damaged sprinklers is an uneven transverse distribution, and it reflects on the quality of the application despite the circumstances in terms of an ideal match of the tank consumption according to the treated surface. The cause of this phenomenon is the deviation of the sprayer from the nominal capacity. The result of increasing or decreasing the capacity of certain sprinklers is stated.

In order to achieve the most precise application of pesticides and complete crop protection, it is not enough that the sprayers have only a modern construction, but it is necessary that they have adequate exploitation potential (Djukic, 2008).

The exploitation potential of the sprayer represents the possibility for reliable execution of the set tasks, when applying pesticides. Exploitation potential implies quantitative and qualitative parameters.

Quantitative parameters include: pump capacity, sprayer capacity, hydraulic mixing device capacity, mutual harmonization of the stated capacities, gear ratio, tank volume, etc.

Qualitative parameters are: precision of dosing and capacity, uniformity of transverse distribution of pesticides, uniformity of droplets in the jet.

The precondition for a quality application is not the possession of a sprayer of a modern concept, but a well-adjusted sprayer that will provide a controlled application of pesticides, and thus the production of health-safe agricultural products. All of the above has influenced the fact that in the previous period, the control of the correctness of the sprayers became a regular obligation in most European countries (Sedlar, 2006). Inspections of sprayers are carried out in accordance with EN 13790. Belgium, Germany and the Netherlands are among the countries that were among the first to introduce mandatory inspections in accordance with this standard. In the area of northern Belgium in the period from September 1995 to December 1998, 17,466 sprayers were tested. Of the tested number of sprayers, 2,802 or 16% of sprayers did not pass the inspection, and therefore their exploitation potential was not satisfactory. Of the 2,802 sprayers, 2,409 (86%) did not receive a quality certificate, due to worn nozzles or a faulty manometer (Langenakens and Pieters, 1999). Inspections of sprayers in Germany have shown that the cause of their malfunction in 19% of cases are worn nozzles, then a faulty manometer and wing valves in 13% and 10% of cases, respectively (Rietz, 1998). At the second European symposium dedicated to the

standardization of sprayer control procedures in Europe, held in Germany, a large number of authors point out the special importance of nozzles. Nozzles as the final elements of the sprayer determine the size of the droplets, the shape and angle of the outlet jet, the amount of liquid and the quality of coverage of the treated surface (Djukic, 2001).

The conducted inspections in the territory of Republika Srpska and Bosnia and Herzegovina also indicate the fact that the malfunction and poor exploitation potential are mostly affected by nozzles and faulty manometers (Malicevic, 2012). In BiH a significant problem is the practice of agricultural producers to buy parts of uncertain quality whose only advantage is the low price. A special risk is the choice of nozzles of questionable quality. Especially in such a situation, the positive effect of correctness control comes to the fore, and the aim of this paper is to point out the importance of quality control of nozzles, which are the most common cause of deviations in application. The use of water-sensitive papers (WSP) is a fast and reliable method for assessing the quality of applications, which is the ultimate goal of the research.

MATERIAL AND METHODS

There are 60 different requirements that every plant protection machine must meet regarding inspections of sprayers in accordance with current standards and regulations. However, the most important thing is: pump operation control, manometer correctness control, mixer operation control and nozzle operation control.

Nozzles as key elements, i.e. their condition directly affect the quality of pesticide applications, i.e. the transverse distribution is in direct relation with the condition of the sprayers (Fig. 1). One of the basic parts is the nozzle cartridge that because of the high pressure exposure has its own lifespan, and is being withdrawn from service when it is worn out.

The service life of the sprayer depends on: the material of construction, the height of the pressure used, the condition of the filter and the maintenance.

The most efficient way to determine the condition of the sprayer is to permanently inspection, monitoring and comparing the flow rate of the old sprayer with the new one (same type and size).

Sprayer quality control involves control of the sprayer capacity and control of the transverse distribution of the sprayer (Sedlar, 2005). The "Laboratory for Testing and Calibration of Plant Protection Machines" has a sprayer capacity meter (Fig. 2), which was used in the research. It consists of an adapter for all types of sprinklers and a memory unit for storing 1000 data of measured capacities.

An Agromehanika AGS 440 mounted sprayer, aggregated with an IMT 539 tractor, was used in the experiment. The volume of the sprayer tank is 440 liters, and the working reach is 8 m. Low pressure pump BM 65 from the same manufacturer, piston-membrane type (2 pistons) with a capacity of 65 l/min on 540 t/min, serving 16 nozzles, with quality mixing. Nozzle carriers are

monoconceptions and are equipped with universal flat-jet nozzles, with a working spray angle of 120°, red-marking coding, markings ID 90/120-04. Nozzles are marked so that looking at the nozzle in the direction of movement, the first on the left represents the number 1, and the last on the right represents the nozzle no. 16. The first eight nozzles treat the left side, while the nozzles at positions 9 - 16 treat the right side of the passage.

Pressures used to test the capacity were 3, 4 and 5 bar providing a nominal capacity value of 1,55; 1,80 and 2,02 l/min.

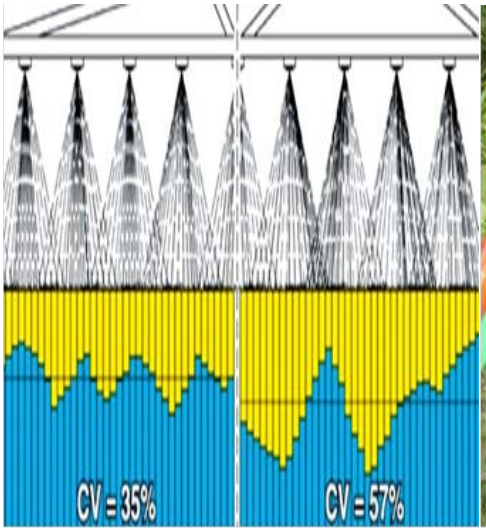


Figure 1. Deviation of transverse distribution
(Source: Internet: http: //www.lechler.de)

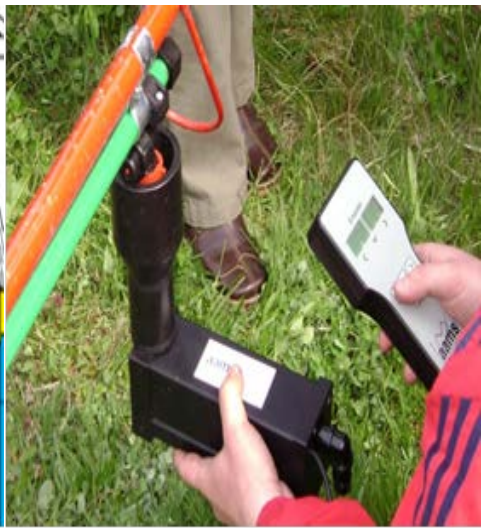


Figure 2. Capacity measurement
(Photo: Malicevic Z.)

An Agromehanika AGS 440 mounted sprayer, aggregated with an IMT 539 tractor, was used in the experiment. The volume of the sprayer tank is 440 liters, and the working reach is 8 m. Low pressure pump BM 65 from the same manufacturer, piston-membrane type (2 pistons) with a capacity of 65 l/min on 540 t/min, serving 16 nozzles, with quality mixing. Nozzle carriers are monoconceptions and are equipped with universal flat-jet nozzles, with a working spray angle of 120°, red-marking coding, markings ID 90/120-04. Nozzles are marked so that looking at the nozzle in the direction of movement, the first on the left represents the number 1, and the last on the right represents the nozzle no. 16. The first eight nozzles treat the left side, while the nozzles at positions 9 - 16 treat the right side of the passage.

Pressures used to test the capacity were 3, 4 and 5 bar providing a nominal capacity value of 1,55; 1,80 and 2,02 l/min.

Sprayer capacity testing included individual capacity measurement and comparison with nominal values. The quality of the application was monitored through the coverage of the WSP, which were set at 16 positions, following the

positions of the sprinklers. The second part of the test included processing, analysis and display of realized capacities in all zones of the spray wing, i.e. deviation from nominal values (Table 1). WSP reading equipment includes the use of high-resolution scanners and processing in the program ImageJ 1.44, which involved determining the coverage of the treated area using appropriate algorithms.

Data on the coverage of plates and the number of drops was recorded on the basis of a passage, and was read using a program ImageJ 1.44. The program was developed by the U.S. National Institutes of Health, and is intended for professional analysis and processing of the photography. After collection and labeling, water-sensitive plates are scanned at high resolution, followed by loading and processing (Zhu, H. et al., 2011; Prodanov, D. Verstreken, K., 2012).

RESULTS AND DISCUSSION

Plant protection with technically right sprayer, and especially the condition of the nozzle, has a crucial influence on the quality of the application. Therefore, it is quite evident that this method of protection will be used also in the future what leads to the fact that pesticides should be used rational and all in order to decrease the quantity of chemical substances to be applied and the number of treatment. Researches in previous years have shown the opposite, frequent infections require a large number of treatments during the year, sometimes up to 20 times (Malicevic, 2010).

The service life, i.e. the period of exploitation of the nozzle, depends on the material of which the nozzle is made. Nozzle resistance to wear and tear represents service life. The nozzle should be withdrawn from operation as soon as the flow increases by 15% or more (GLOBALGAP). Working with nozzles that have flow increase of 15% and more than 15%, represents loss both in terms of economy and quality of treatment and protection of the human environment (Djukic, 2008).

In practice, operators are in a dilemma when the sprayer should be withdrawn from use, i.e. when the flow has increased by more than 15%. Due to all the above, nozzles 3, 7 and 8 on the left side meet the condition to be withdrawn from operation due to increased flow. On the right side of the device, nozzles 11, 12, 13, 15 should also be withdrawn from service. Nozzles with reduced flow relative to nominal values should be cleaned and returned to service. The cause of the reduced capacity is mechanical impurities that can occur due to the use of poor quality water. There is a possibility of impurities in the preparation as well as sawdust due to friction of the working assemblies of the device.

There are two ways to reduce the possibility of exploiting nozzles that have exceeded the tolerance to increase the flow: The first way, is to control the nozzles after a certain time of use, e.g. 30 hours of operation. Another way is to purchase nozzles that have been tested by an authorized laboratory and that have

the appropriate quality certificate from which it is clearly seen and after how many hours of operation the flow increases by 15%.

Table 1. Sprayer capacity and deviations from nominal values

	P (*bar*)	Nozzle position															
		Left side, capacity (*l/min*)								Right side, capacity (*l/min*)							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
capacity	3	1,29	0,34	2,06	1,55	0,26	0,43	2,06	1,89	1,12	1,29	2,41	2,41	2,49	1,46	2,23	1,20
	4	1,50	0,40	2,40	1,80	0,30	0,50	2,40	2,20	1,30	1,50	2,80	2,80	2,90	1,70	2,60	1,40
	5	1,68	0,45	2,69	2,02	0,34	0,56	2,69	2,47	1,46	1,68	3,15	3,14	3,25	1,91	2,92	1,57
capacity	3	-16,77	-78,06	+31,00	0,00	-83,22	-72,25	+32,90	+21,93	-27,74	-16,77	+55,48	+55,46	+60,64	-5,80	+43,87	-22,58
	4	-16,66	-78,06	+31,00	0,00	-83,22	-72,25	+32,90	+21,93	-27,74	-16,77	+55,48	+55,46	+60,64	-5,80	+43,87	-22,58
	5	-16,83	-77,72	+33,17	0,00	-83,16	-72,27	+33,16	+22,27	-27,72	-16,83	+55,44	+55,42	+60,89	-5,44	+44,55	-22,27
deviation	3	-0,26	-1,21	+0,51	0,00	-1,29	-1,12	+0,51	+0,34	-0,43	-0,26	+0,86	+0,86	+0,94	-0,09	+0,68	-0,35
	4	-0,30	-1,40	+0,60	0,00	-1,50	-1,30	+0,60	+0,40	-0,50	-0,30	+1,00	+1,00	+1,10	-0,10	+0,80	-0,40
	5	-0,34	-1,57	+0,67	0,00	-1,68	-1,46	+0,67	+0,45	-0,56	-0,34	+1,12	+1,11	+1,23	-0,11	+0,90	-0,45
deviation	3	9,88 (*l/min*) (- 20,32 %)								14,61 (*l/min*) (+17,82 %)							
	4	11,50 (*l/min*) (- 20,13 %)								17,00 (*l/min*) (+18,05 %)							
	5	12,90 (*l/min*) (- 20,17 %)								19,07 (*l/min*) (+ 18,00 %)							
coverage	3	26,6	10,5	49,7	37,8	5,60	13,3	53,2	41,3	14,7	23,8	51,8	53,9	57,4	30,1	39,9	20,3
	4	25,8	12,3	52,1	32,2	8,4	23,1	57,2	25,3	18,5	33,6	57,1	56,6	54,5	40,2	31,1	25,6
	5	31,2	26,8	41,1	19,8	32,2	39,5	49,4	19,8	28,9	42,7	50,2	49,3	56,8	45,6	39,5	33,2
coverage	3	29,7 (%)								36,5 (%)							
	4	30,0 (%)								39,8 (%)							
	5	32,5 (%)								43,2 (%)							

„+“increase ; „-“ decrease

Despite criticism of the excessive use of chemical plant protection products, farmers will continue to use and apply sprayers. If it is not possible to influence the use of chemicals to such an extent, the efficiency of the application can be increased through the control of transverse distribution. Good setting of the sprayer increases the quality and efficiency, which is manifested in better deposition of the agent and uniform application, and prevents inadequate and increased use of pesticides (Visacki, 2015).

The research aims to show that based on the presented parameters (total capacity of the device) the ideal usage of the agent per surface is obtained, while significant deviations from the aspect of nozzles flow are recorded, i.e. to indicate the importance of transverse distribution (Fig. 1). This can be the cause of problems with the application of pesticides, and is reflected in the fact that certain zones receive more funds, and others less. In the survey, only one nozzle meets the allowable deviation from the nominal flow value.

Measured values of the sprayer capacity expressed in liters per minute are shown in table 1. Values of measured capacities (3, 4 and 5 bar) show significant deviations, both in the form of increase and decrease from the nominal value of capacity. In accordance with the current standards that regulate this area, which require withdrawal from operation in case of capacity increase by 15% and more, it is clear that seven nozzles do not meet the requirements of the standard. The table shows the quantities (l/min) as well as percentage deviations of the sprayer according to the positions, as well as deviations of the device capacity towards the sides. Positions of nozzles whose capacity exceeds the allowed deviation are: 3, 7, 8, 11, 12, 13 and 15. Nozzles 3, 7 and 8 they are on the left side of the device while the others are on the right side of the device. The largest deviation of 60.89% in terms of capacity increase was recorded at the position of the nozzle number 13, which is located on the right side of the device. Nozzles at positions 11 and 12 also have an increased capacity of 55.46%, which is high above the allowable deviation limit. The smallest deviation of 22.27% in terms of capacity increase was recorded at position 8. The coefficient of variation is particularly high and ranged from 48,31% (p=4 bar) to 48,74 % (p=5 bar), which can be considered very bad.

By concentrations in nuts, in most cases, the metals were arranged as the following diminishing series: Fe > Pb > Zn > Mn > Cr > Cd. Published data on the content of metals in nuts around the world are presented in Table 3.

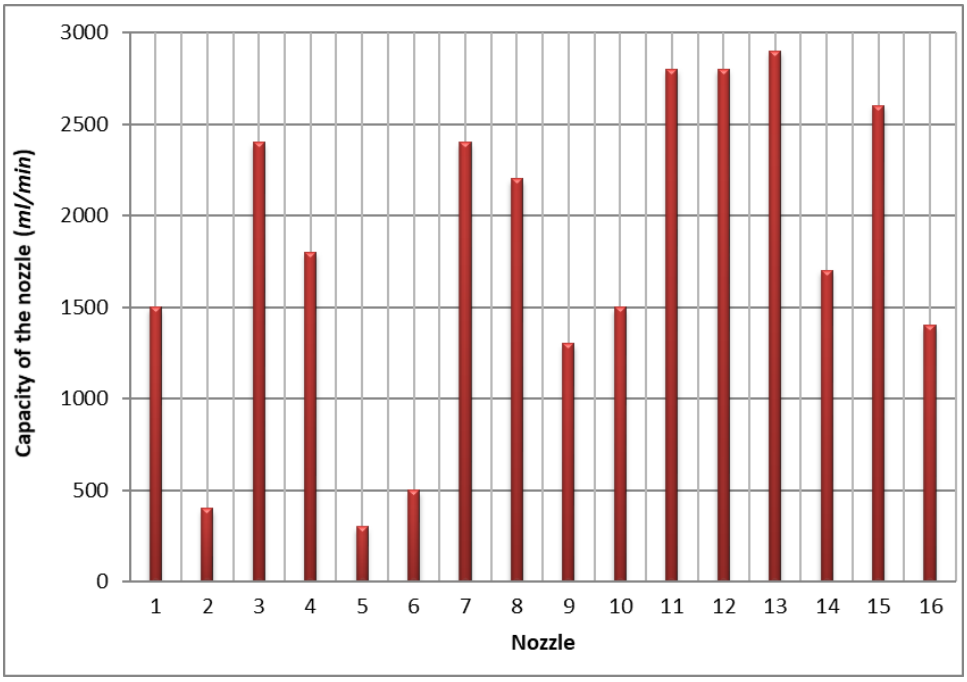
The analysis of the obtained values of the tests shows a large deviation according to individual positions. In practice, this is a serious and common problem due to the fact that according to the form for calculating the standard, the average deviation is only 1.11%, and the individual deviation by position is over 60%. The analysis of the data from Table 1 measured the deviation towards the sides, which ranges from 17.82% in the form of an increase in capacity on the right, or a decrease in capacity of 20.32% on the left side of the device.

This is a common problem in which a large number of agricultural producers are found, i.e. as an indicator of the adjustment of operating parameters, they monitor the consumption of liquid according to the treated area, i.e. the consumption of preparations according to the sprayer tank. Deviation in the capacity of the nozzle is the cause of poor pesticide application, i.e. reflected in the uneven coverage of the treated area (fig. 3).

The average coverage at p=3 bar was 33,10%, at p=4 bar was 34,91% and 37,83% at pressure of p=5 bar, which is the best result in research.

The coefficient of variation is also extremely high, and the best result can be considered 29.26% at a pressure of 5 bar, while at the other two pressures they are significantly worse.

Overcoming this problem lies in the fact that the control of the nozzle capacity eliminates the possibility of poor transverse distribution, reduces the coefficient of variation, and that the consumption according to the treated area fits perfectly.



Graph 1. Capacity of inspected nozzles (p=4 bara)

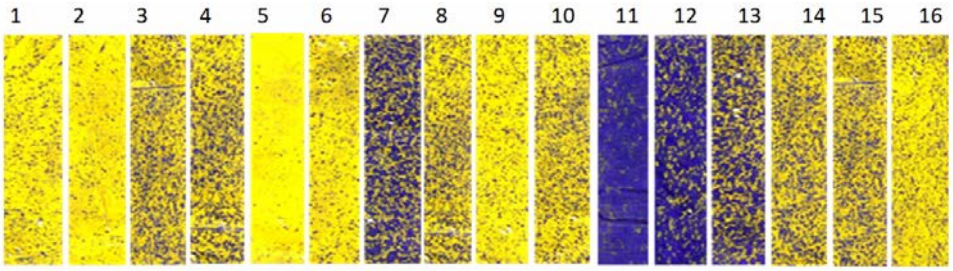


Figure 3. Coverage of water-sensitive papers (*p*=4 *bar*)
(Photo: Malicevic Z.)

The control of the nozzle capacity provides a precondition for the controlled application of plant protection products, and the possibility of deviations in terms of capacity according to the positions of the nozzle is excluded.

CONCLUSIONS

The quality of application from the aspect of pesticide application devices cannot be characterized as satisfactory. A major problem in practice is the untrainedness of the operator of the application devices and the lack of basic knowledge in terms of adjusting the operating parameters that provide the norm of treatment.

Treatment with inspected sprayer has multiple advantages over uncontrolled, and is reflected in: better uniformity in terms of transverse distribution, more uniform and better coverage of plant parts, lower usage of pesticides, increased performance of human and machine labor and lower cost of protection.

Inspection of the device, and especially the capacity of the nozzle, provides a model of controlled application of pesticides, which not only provides optimal consumption of pesticides but also improves the effect of transverse distribution. The tested capacity of the nozzle as well as the speed of movement enable the reduction of the norm and the dose of treatment, increases the precision of the device itself, and thus significantly increases the degree of protection of the human environment.

Nozzle capacity control is a reliable indicator of the quality of transverse distribution, and at the same time prevents the possibility of inaccurate application, with optimal consumption of the product according to the surface.

The conducted research and analysis indicate the necessity of regular control of the nozzle capacity, because the quality of the application largely depends on their condition.

Not enough attention is paid to the control of transverse distribution; however, these tests have shown that it is perhaps the most important segment in the quality and precise application of pesticides. Quality application implies control of transverse distribution, which is certainly a step towards optimizing the use of pesticides.

REFERENCES

Djukic, N., Sedlar, A., Bugarin, R. (2008): Defining a mathematical model of nozzle consuption and its significance for domestic production. Modern agricultural technology, 34, 1-2, 63-71.

Djukic, N., Sedlar, A. (2001): Good sprayer, effective protection. Review of agronomic knowledge, 6, 48-50.

Langenakens, J., Pieters M. (1999): Organization and Results of The Compulsory Inspection of Sprayers in Belgium. 7th International Congress on Agrcultural, Adana-Turkiye, 50-53.

Malicevic Z, Railic B, Mitric S, Babic M, Marceta I,. Improvement measures of the sprayers efficiency in order to apply small and medium norms by spraying orchards, Agroznanje, Banja Luka (2010), vol.11, br. 4., 135 – 140.

Malicevic, Z., Railic, B. Mitric, S., Mihajlovic, D., Babic, M. (2012): "Techniques for pesticide application in order to obtain GlobalGAP standards in the Republic of Srpska", Agroknowledge, vol. 13., br. 3., 421-430 str., Conference of agronomists of Republika Srpska.

Prodanov, D., Verstreken, K. (2012): Automated Segmentation and Morphometric of Cell and Tissue Structures. Selected Algorithms in ImageJ, In tech open sciens/open minds, Molecular Imaging, March 16th, 183 – 208.

Rietz, S., Gamzlemeier, H. (1998): Inspection of plant protection equipment in Europe, AgEng, Oslo, 98-A-023.

Sedlar, A. (2006): Analysis of sprayer testing methods, Master's thesis, Novi Sad.

Sedlar, A., Djukic, N., Bugarin, R. (2005): The importance of regular sprayer inspection; Journal of Agronomic Knowledge, Novi Sad, br. 4

Visacki, V., Sedlar, A., Turan, J., Bugarin, R., Burg, P. (2014): Uniformity of pesticide application at the most common exploitation parameters. Modern agricultural technology, 40, No. 3, 125-134.

Visacki, V., Lazic, S., Sunjka, D., Sedlar, A., Bugarin, R., Turan, J., Burg, P. (2015): Determining the quality of application of field sprayer in corn, 41, No. 1, 57-66.

Zhu, H., Salyani, M., Fox, R.D. (2011): A portable scanning system for evaluation of spray deposit distribution, Computers and Electronics in Agriculture, 76: 38–43.

Koprivica, R., Veljković, B., Radivojević, D., Dedić, T., Mileusnić, Z., Đokić, D. (2020): Equipment of family farms with agricultural mechanization in Northwest of Montenegro. Agriculture and Forestry, 66 (3): 231-239.

DOI: 10.17707/AgricultForest.66.3.19

Ranko KOPRIVICA, Biljana VELJKOVIĆ [1], Dušan RADIVOJEVIĆ [2], Tatjana DEDIĆ [3], Zoran MILEUSNIĆ [2], Dragoslav ĐOKIĆ [4]

EQUIPMENT OF FAMILY FARMS WITH AGRICULTURAL MECHANIZATION IN NORTHWEST OF MONTENEGRO

SUMMARY

The paper presents the results of testing equipment at family farms with agricultural machinery in Northwest Montenegro in municipalities Pljevlja, Žabljak, Kolašin and Mojkovac. In the mentioned municipalities, 123 agricultural farms in 65 villages were surveyed. The total area of available land on farms is 1612.08 ha, of which only 8.27% are cultivated and the rest meadows and pastures. The average area of family farms is 13.11 ha of used agricultural land, which is three times more than the average in Montenegro. Single-axle tractors with an engine power of 5-10 kW are represented by 16% of the total number of tractors. Family farms are not sufficiently equipped with two-axle tractors, because every other one has a tractor (0.51 tractors per farm). The tractors are average power of 30.12 kW. Energy equipments in the surveyed area, expressed through the nominal engine power of two-axle tractors per unit area, in averages at 1.18 kW / ha.

There's a shortage of tractors with engine power over 50 kW with rear and front wheel drive (double traction). Family farms are insufficiently equipped with attached machines (1.28 machines / farm). Ratios per one two-axle tractor are 2.49 attachments and 10.27 ha of used land. There is a lack of machines for vertical tillage and combined machines for pre-sowing soil preparation (chisel plows, seed drills, rotary harrows), as well a seeder for small grains and corn. In addition, there is a lack of machines for the preparation of silage and haylage in silage facilities (self-loading trailers and combines), as well roll presses, wrappers and catchers wrapped in foil.

Keywords: size, equipment, farms, tractors, attachments, energy equipment.

[1]Ranko Koprivica, Biljana Veljković *(corresponding author: biljavz@kg.ac.rs), University of Kragujevac, Faculty of Agronomy in Čačak, Cara Dušan 34, 32000 Čačak, SERBIA.
[2]Dušan Radivojević, Zoran Mileusnić, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade Zemun, SERBIA.
[3]Tatjana Dedić, FAO-Regional Office for Europe and Central Asia, Budapest, HUNGARY.
[4]Dragoslav Đokić, Institute for forage crops, Globoder 37251 Kruševac, SERBIA.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Recieved:17/06/2020 *Accepted:24/09/2020*

INTRODUCTION

Sustainable functioning of agricultural family farms depends on their equipment with agricultural machinery, but also on the available human labor. Unfortunately, the working age population, especially the younger are increasingly missing in the countryside. It is necessary to facilitate and mechanize work processes and the application of agro-technical measures.

Rural aging is a common occurrence and is found in many countries, regardless of economic development (Veljković et al. 2020). In Montenegro, the labor force is becoming a limiting factor in the development of agriculture and revitalization of family farms, because 44% of the total number of people working in agriculture is older than 55 years (Šarović, 2014). Thus, the intensive development of agriculture is largely limited by the insufficient equipment of family farms with agricultural machinery and the lack of labor force. According to the 2010 census in Montenegro, agricultural production takes place mainly on 5265 family farms, on an area of 294400.7 ha of available land (Statistical Office of Montenegro, 2011). In the examined area, family farms are engaged in livestock production, while crop production is mainly for the needs of animal feed (meadow hay and cereals).

The aim of the research is to determine the equipment of family farms with agricultural mechanization based on the analysis of the existing situation and to give recommendations for the necessary mechanization.

MATERIAL AND METHODS

The survey of agricultural family farms was conducted in the Northwest part of Montenegro in the territory of municipalities Pljevlja (41 willages, 71 farms), Žabljak (14 willages, 16 farms), Kolašin (2 willages, 18 farms), and Mojkovac (8 willages, 18 farms). Villages and farms were selected for the survey by random sampling. In this 4 municipalities, a total of 123 agricultural farms in 65 villages were surveyed. The researchers used the interview questionnaire to record data on farm size, available areas (arable land, meadows and pastures), number, power, age, types and manufacturers of tractors, as well as the number and type of attachments. After the survey, the data were grouped, processed and presented in tables and graphs. The technical equipment of farms with the effective power of tractors per unit of agricultural area in the surveyed area is defined by the expression:

$$Et = \frac{\sum_{i=1-n}^{n} Pe(i)}{\sum_{i=1}^{m} Ai} \ (kW/ha)$$

Et – equipment of farms with tractor power per agricultural area
A (ha) – agricultural area
Pe (kW) – effective tractor engine power

In addition to the results of the survey, the paper also used statistical data from the Statistical Office of Montenegro, Document of Cenzus of Agriculture

2010, and Annual statistics of transport, storage and communications 2011-2019. Based on the data collected in the field, the equipment of family farms with mechanization was registered. The number of tractors per farm, arable land per tractor, average tractor power, effective power per hectare, equipment of farms with attachments, number of attachments per tractor and area of used land per attachment were determined.

RESULTS AND DISCUSSION

The investigated area is the Northwest part of Montenegro and occupies 63359.9 ha or 21.52% of the total area of available land, so that one fifth of agricultural production takes place in this part of Montenegro. In all four municipalities, pastures and meadows cover an area of 45951.1 ha or 72.52% of the total land (Table 1). The configuration of the terrain is very diverse, it consists of river valleys, hills, and mostly hills and mountains, so less than 2% of the available agricultural land is cultivated.

Table 1. Land according to the manner of use on family farms by municipalities

Municipality	Total available land ha	Total utilized agricultural area ha			Orchards and Vineyards	Permanent meadows and pastures ha
		All	Arable fields, gardens	Utilised arable land		
Žabljak	9610	7480	6.6	57	0.0	7416.9
Kolašin	14242	8269	116.3	41.4	4.6	8411.7
Mojkovac	10144.8	7873	44.6	29.7	7.5	7779.5
Pjevlja	29363.1	22901.4	159	280	74.4	22343
Total ha for the surveyed area	63359.9	46523.4	326,5	408.1	86.5	45951.1
Share %	21.52	21.87	13.53	11.16	4.99	22.48
Total ha Montenegro	294400.7	212724.4	2412.8	3656.4	1734	204359.4

(Source: Statistical Office of Montenegro 2011, p.107-121)

Table 2. Land areas of surveyed family farms by municipalities

Municipality	Number villages	Number farms	Available land in ha	Arable land		Average farm size in ha
				ha	%	
Pjevlja	41	71	1.044.67	93.29	8.93	14.71
Žabljak	14	16	288	10.95	3.8	18.00
Kolašin	2	18	137.96	11.93	8.65	7.66
Mojkovac	8	18	141.45	17.20	12.16	7.86
Total:	65	123	1612.08	133.37	8.27	13.11

(Source: Own research)

Family farms organize production on area 1612.08 ha. The average area of used agricultural land per surveyed farm is 13.11 ha, which is three times more than the average in Montenegro (4.60 ha). The farms with the largest average area are in the mountainous parts of the municipality of Žabljak 18 ha (Table 2). Some family farms in the hilly and mountainous area own over 100 ha of land. These are mostly summer pastures on katuns, which according to the research of Šarović (2014) are over 58% in Northern Montenegro. Farmers in the surveyed area by average land are ahead than farmers in Slovenia, Greece, Serbia, Croatia, Western Turkey where the average farm area is less than 10 ha, and behind Poland, Denmark (Poje, 2016, Koprivica et al. 2009, Radivojević, 2014, Juscinski et al. 2017, Ozpinar, 2020).

The available agricultural land on farms is little cultivated 8.27% or 133.37 ha and these are mostly gardens and fields in river valleys, on hills and mountain plateaus.

According to the 2010 Census of Agriculture, 5,265 family farms in Montenegro owned 1,654 single-axle tractors up to 10 years of age and 4,036 single-axle tractors over 10 years of age (1.1 single-axle tractors per farm). The total number of two-axle tractors is 4560, of which 123 are owned by business entities (companies, cooperatives, etc.), which is half less (228) than in 1991 (Perošević, 2020). The age structure of tractors is unfavorable, as 52% of tractors are older than 20 years, and 8.4% of tractors are younger than 10 years. Most two-axle tractors have an engine power of 30-60 kW (3522) or 77.23% of the total number. A more precise picture of the number of tractors in Montenegro is given by the fact that in the period from 2013 to 2017, 866 tractors were registered. Registered tractors are older than 25 years, 55.6%. It is positive that tractors up to 5 years of age are represented with 38.16%. Poje (2016) states that 91.8% of registered tractors in Croatia are older than 10 years. The average age of registered tractors is 28.44 years, and in Osijek-Baranja County 20.71 years (Zimmer, 2019). In Slovenia, out of the total number of registered tractors in 2014, 83,291 are older than 12 years. The average age of registered tractors is more than 21 years (Poje, 2016). In Hungary, the average age of tractors in 2013 was 18.3 years, and in 2016 it was around 12 years. Of all tractor categories, 57% are older than 10 years. Tractors under the age of 10 are mostly tractors with a power of more than 60 kW (Kesmarki-Gally and Rak, 2018). In Serbia, in 2010, 95% of tractors were older than 10 years (Radivojević, 2014). In Turkey, 46% of the total number of tractors is older than 25, while in the Western part of Turkey, 88% of tractors are younger than 20 (Ozpinar, 2020).

For easier comparison and assessment of the level of equipment of farms with agricultural machinery, the number of single-axle and two-axle tractors and energy supply per unit area are shown (Tables 3 and 4). In the surveyed area, single-axle tractors with a power of 5-10 kW are 12 -16% of the total number of tractors. In addition to single-axle tractors, the farms also have 63 two-axle tractors, which make up 84% of the total number of tractors (Table 3).

Family farms are not sufficiently equipped with two-axle tractors, because on average, every other farm has a tractor (0.51 tractors per farm). According to the number of tractors per farm, this is more than Hungary (0.25) and Kosovo (0.43), and less than Serbia (0.64), Poland (0.77), Turkey (0.99), Slovenia (1.47), as well as the Croatian Osijek-Baranja County (3.3) (Koprivica et al. 2010, Radivojević, 2014, Poje, 2016, Juscinski et al. 2017, Kesmarki-Gally and Rak, 2018, Zimmer, 2019, Ozpinar, 2020).

Table 3. Number and installed power of tractors on family farms in the surveyed area

Municipality	Number of tractor			Total installed tractor power kW		
	Single-axle	Two-axle	Total	Single-axle	Two-axle	Total
Pljevlja	5	51	56	38.5	1527.5	1.566
Žabljak	3	5	8	38	128.5	166.5
Kolašin	2	3	5	10.3	95.0	105.3
Mojkovac	2	4	6	9.4	146.5	155.9
Total	12	63	75	96.2	1897.5	1993.7

(Source: Own research)

The average energy equipment in the surveyed area is 1.18 kW / ha, the highest in Pljevlja is 1.46 kW / ha, and the lowest in Žabljak is only 0.45 kW / ha. For comparison, in Vojvodina, the energy equipment in the private sector is 3.54 kW / ha, and on one tractor with an average power of 40 kW is 15.83 ha of agricultural land. The use of tractor mechanical power in America is 0.783 kW / ha, in Europe 0.694 kW / ha, in Turkey 2.42 kW / ha, in Kosovo 2.55 kW / ha, in Poland 4.9 kW / ha (Koprivica et al. 2010, Bahattin, 2013, Juscinski et al. 2017). In the southeast part of Hungary, farms up to 4 ha in size are equipped with 1.04 tractors / farms, with an average power of 35.9 kW, 5.08 ha each and 6.86 kW / ha. The farm of 8.1-16 ha has 1.5 tractors with an average power of 84.1 kW for processing 13.26 ha and energy equipment with 3.6 kW / ha (Baranyai et al. 2014).

Table 4. Energy equipment of family farms with tractors in the surveyed area

Municipality	Average tractor power	Energy equipment kW/ha	Number ha/ tractors	Number of tractors / per farm
Pljevlja	29.95	1.46	20.48	0.72
Žabljak	25.57	0.45	57.60	0.31
Kolašin	31.67	0.69	45.98	0.17
Mojkovac	36.62	1.03	35.36	0.22
Average	30.12	1.18	25.58	0.51

(Source: Own research)

In the examined area, the average two-axle tractor with a power of 30.12 kW cultivates 25.58 ha of used land, which is a large area for tractors of this category. In Slovenia there is 4 ha per tractor, in Croatia 5.32 ha, in Poland 6.35 ha, in Serbia 7.13 ha, in Kosovo 14.59 ha, in Turkey 17.78 ha of used land (Poje, 2016, Koprivica et al. 2010, Radivojević, 2014, Juscinski et al. 2017, Bahattin, 2013).

In the survey, two-axle tractors were divided into 4 categories according to engine power (Figure 1). In terms of the number of tractors, are mostly tractors with rear-wheel drive up to 30 kW (73.02%), among which the IMT 539 tractor dominates with 60.32% of the total number of two-axle tractors. Then are the tractors of the power category of 31-40 kW (19.05%) to which the tractors IMT-542 (17.46%) and Ursus 3512 (1.59%) belong. There is the least tractors with drive four all-wheel (double traction) with engine power over 50 kW (3.17%), one tractor each IMT 577 and Rakovica 75 (Figure 1 and 2).

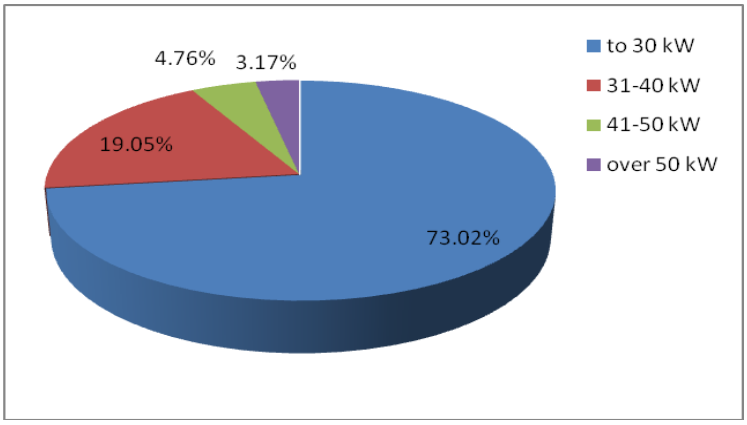


Figure 1. Two-axle tractors by category in farms

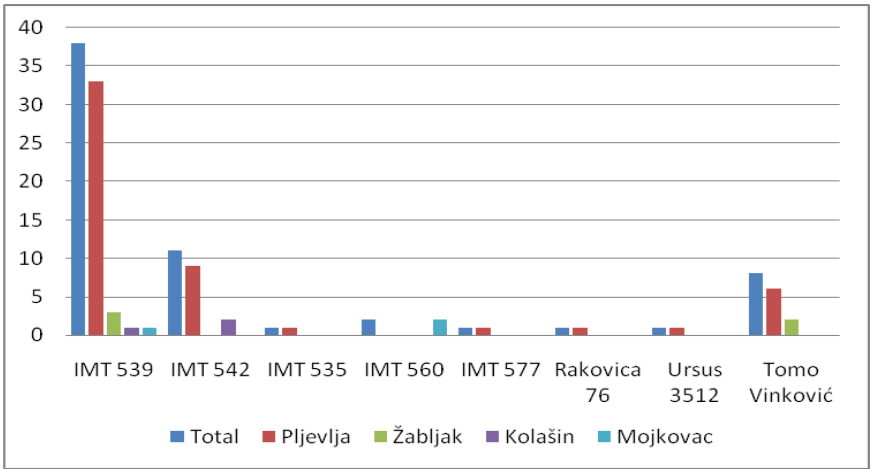


Figure 2. Tractors by types and manufacturers in the surveyed municipalities

For comparison, in Serbia there are the most tractors of 19-37 kW (61%) and 37-66 kW (31%) (Radivojević, 2014). In Poland, of the total number of tractors, 31% is engine power up to 50 kW, and the same percentage of tractors is over 50 kW (Juscinski et al. 2017). In Hungary, the number of 60 kW tractors has increased 4.5 times, but tractors up to 59 kW still predominate on farms (Kesmarki-Gally and Rak, 2017). Tractor sales in Slovenia in 2014 were the same for tractors with a power of 40-60 kW (33%) and 60-80 kW (34%) of the total number of tractors sold in that year (Poje, 2016). In Turkey, according to Bahattin et al. (2013) is a tractor engine power of 36-51 kW (37.55%) of the total number of tractors.

The Ministry of Agriculture and Rural Development of Montenegro, through EU funds, provided non-refundable funds for investments in equipping farms through projects. Approximate number of new tractors first time registred and producead in the previous and current calendar year of registration in period 2011-2019 are 818.

In the surveyed area, some farmers used these funds and procured new tractors Ursus, Belarus, Foton, Mahindra, Tafe, which were not included in the research results, because they were procured after the survey.

In addition to the number of tractors and their power, the level of mechanization also determines the number of attached machines on farms.

Table 5. Equipment with attached machines on family farms by municipalities

Municipality	Total number attached machines	Number attached machines on farms	Number ha per attached machines	Number attached machines per tractor
Pjevlja	105	1.48	9.95	2.06
Žabljak	14	0.88	20.57	2.80
Kolašin	15	0.83	9.20	5.0
Mojkovac	22	1.22	6.43	5.5
Total /Average	157	1.28	10.27	2.49

According to statistical data in Montenegro, family farms have 8196 attachments for two-axle tractors. The most have trailers 57% and tillage machines, while have less mowers 17%, presses hay 12.63%, and the least of sowing and planting machines 3.11%

The family farms covered by the research have 157 different attachments for two-axle tractors. Most plows are 23.57% and trailers 22.93% of the total number of attached machines. The tractor is mostly used for transport, and less in tillage, because only 3.59 ha of arable land is plowed with one plow. Harrows, smaller rotary cultivators, disc harrows, rollers are mostly used for pre-sowing soil preparation, and only 1 seedbed cultivator is used (Tables 5 and 6).

From the machines for harvesting fodder plants, the farmers own 18 tractor mowers, 9 hay collectors and 12 hay presses. Every other surveyed farm owns one of the mowers (motor or tractor mower).

Table 6. Number of tractor attachments by types in the surveyed area by municipalities

Type of machine	Pjevlja	Žabljak	Kolašin	Mojkovac	Total
Plow	22	3	5	7	37
Harrow	17	-	4	4	25
Roller	3	-	-	1	4
Tractor trailers	23	5	3	5	36
Disc harrows	1	1	-	1	3
Sediment speader	3	-	-	-	3
Manure spreader	3	1	-	-	4
Haymaker	5	-	1	3	9
Tractor mowers	16	2	-	-	18
Cutter	4	-	-	-	4
Seedbed cultivator	1	-	-	-	1
Square beler	7	2	2	1	12
Total	105	14	15	22	157

In this area, farmers have an average of 1.28 tractor attachments per farm, which is close to the national average. On average, one two-axle tractor with a power of 30.12 kW cultivates 10.27 ha of available land with 2.49 attached machines. In the Northeast part of Montenegro are 2.49 machines per two-axle tractor, 2.67 in Kosovo, 5.89 in Serbia, 7.26 in Turkey (Koprivica et al. 2009, Koprivica et al. 2010, Radivojević, 2014, Ozpinar, 2020).

CONCLUSIONS

Family farms are not sufficiently equipped with two-axle tractors (0.51 tractors per farm), which is below the national average (0.87 tractors per farm). Two-axle tractors with rear and front-wheel drive (double traction) over 50 kW is generally missing. The equipment of tractors with attachments is very poor, because there are 2.49 attachments on 10.27 ha of used land per one two-axle tractor with a power of 30.11 kW. In the surveyed area, and in the whole of Montenegro, there is a lack of combined machines for rational basic tillage and pre-sowing soil preparation: chisel plows, combined machines (seedbed cultivator), medium-heavy disc harrows and rotary harrows, as well as seeders for small grains and corn. In addition, there is a lack of tractor mowers with a double cutting machine, hay collectors and tedders, self-loading trailers, forage harvesters, roll presses, wrappers and balers for silage and haylage.

ACKNOWLEDGEMENTS

The research supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project Ref. No. TR.31051.

REFERENCES

Bahattin A. (2013): Agricultural mechanization in Turkey. IERI Procedia 5 (1)41-44.

Baranyai Z., Szabo G., Vasary M. (2014): Analysis of machine use in Hungarian agriculture - Is there any future for machinery sharing arrangements. Roczniki (Annals), Polish Association of Agricultural Economists and Agribusiness. Stowarzyszenie Ekonomistow Rolnictwa e Agrobiznesu (SERiA) vol. XVI (3) p. 24-29

Juscinski S., Piekarski W., Chomik Z. (2017): Analysis of Power Equipment Level on the Example of Farm Tractors in Selected Farms of Przeworsk Province. Agricultural engineering, Vol. 21, No.3, pp. 17-27.

Kesmarki-Gally S. E., Rak R. (2018): Challenges for the development of agricultural power machines and their operating costs in Hungary. Roczniki Naukowe SERiA XX (5) p. 81-86.

Koprivica R., Veljković B., Thaqi A., Sharku A. (2010): Opremljenost porodičnih farmi poljoprivrednom mehanizacijom na području Šar Planine. Traktori i pogonske mašine, Vol. 15 No 4, p. 51-58. Novi Sad.

Koprivica, R., Veljković B., Dedić T., Martinov, S. (2009.): Analiza obezbeđenosti traktorima na porodičnim gazdinstvima u području Severoistočne Crne Gore. Traktori i pogonske mašine, Vol. 14, No 5, p. 23-28, Novi Sad.

Ozpınar, S. (2020): Mechanization and agricultural farm structure in the agricultural area of the Dardanelles region. Int. J. Agric. Environ. Food Sci., 4(1), 39-56.

Perošević, N. (2020): Agriculture development in the Municipality of Nikšić (1945-1991). Agriculture and Forestry, 66 (1): 83-94.

Poje T. (2016): Stanje traktorske tehnike na Slovenskim obiteljskim gospodarstvima. 51.Hrvatski i 11. međunarodni simpozij agronoma, 15.-18. veljače 2016. godine, Opatija, Hrvatska, Zbornik radova 2016 pp.498-501.

Radivojević D. (2014). Poljoprivredna mehanizacija oprema i objekti, ISBN 978-86-6161-111-7, COBISS.SR-ID 206984204 CIP 631.3 (497.11) "2012" (083.41), Zavod za statistiku Republike Srbije, Posebna publikacija - Monografska studija p.p. 1-154.

Statistical Office of Montenegro (2011): Structure of agricultural holdings Agricultural census 2010. Podgorica

Statistical Office of Montenegro (2019): Annual statistics of transport, storage and communications https://www.monstat.org/userfiles/file/saobracaj/2019/PUBLIKACIJA-%20GODISNJA%20STATISTIKA%20SAOBRACAJA%202019-cg(1).pdf

Šarović R. (2014): Agricultural family farm in Montenegro. Agriculture & Forestry, Vol. 60. Issue 1: 145-156.

Veljković B., Koprivica R., Milošević T., Radivojević D., Broćić Z. (2020): Udruživanje u funkciji održivog ruralnog razvoja. Agroekonomika godina 49 br. 86 p. 1-11.

Zimmer D. (2019): Optimalno opremanje poljoprivrednih gospodarstava sredstvima poljoprivredne mehanizacije. Doktorska disertacija. Fakultet agrobiotehničkih znanosti Osijek Sveučilište Josipa Jurja Strossmayera u Osijeku.

Popović, T., Matijašević, S., Raičević, D., Mijović, S. (2020): Influence of vine loads with fertile buds on grape yield and quality of variety Žižak in Podgorica vineyard area. Agriculture and Forestry, 66 (3): 241-250.

DOI: 10.17707/AgricultForest.66.3.20

Tatjana POPOVIĆ[1], Saša MATIJAŠEVIĆ[2], Danijela RAIČEVIĆ, Slavko MIJOVIĆ[1]

INFLUENCE OF VINE LOADS WITH FERTILE BUDS ON GRAPE YIELD AND QUALITY OF VARIETY ŽIŽAK IN PODGORICA VINEYARD AREA

SUMMARY

Results of a two - year study of the influence of different vine loads with fertile buds on elements of grape yield and quality of Žižak grape variety are presented in this paper. The research was conducted in the Podgorica sub region, at the Lješkopolje site. Four different vine loads with fertile buds were applied: 14 buds, 18 buds, 20 and 24 buds per vine. The results of this research show that the applied loads significantly influence the examined parameters of the autochthonous variety Žižak. The highest percentage of developed and fertile shoots, as well as the average number of flowers per bud, had a variant with load of 14 buds. The relative coefficient of yielding in all examined load variants had mean values. In terms of the number of flowers per fertile shoot in the two-year average, variants with a load of 18 and 14 buds (1.44 and 1.39) stood out. The highest yield of grapes in the two-year average had the variant with a load of 24 buds - 3.60 kg/vine, while the highest bunch weight had the variant V1-185 g. The quality of grapes also varied significantly under the influence of the vine load with fertile buds. The highest sugar content in must had variant with a load of 18 buds (22.32%), while the highest acid content (6.27g/l) was recorded in the variant with 24 buds per vine.

Keywords: Žižak, load, fertility, yield, bunch weight, grape quality

INTRODUCTION

Pruning is an ampelotechnical measure that should enable the achievement of maximum yields of grapes, without reflecting on the weakening of the vegetative power of the vine and deterioration of quality of grapes (Cindrić et al., 2000). This ampelotechnical measure seeks to ensure balance between vegetative strength and condition of vine and the quality of grape yield, in a manner that does not jeopardize productive capacity of the vine during decades of vineyard exploitation (Mijatović et al., 2018). Pruning maintains the cultivation form of the

[1]Tatjana Popović (corresponding author: tatjanapopovic@t-com.me), Danijela Raičević, Slavko Mijović, Biotechnical faculty Podgorica, MONTENEGRO.
[2]Saša Matijašević, Faculty of Agriculture Belgrade, SERBIA .
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Recieved:24/08/2020 *Accepted:28/09/2020*

vine, regulates the vegetative and fertile potential, and indirectly affects the quantity and quality of grape yield (Mirošević et al., 2008).

Proper loading of vines with fertile buds is achieved on the basis of knowledge on the vegetative potential of vines, agrotechnical growing conditions and the quality of grape yield that is to be achieved (Vujović, 2013). The optimal load of vines with fertile buds for each variety must be in accordance with its biological characteristics. Leaf size and leaf area, movement and yield of buds, shoot growth, yield, grape quality, etc. largely depend on the optimal load of vines with fertile buds (Pejović, 1982). Therefore, in each vineyard region, it is necessary to establish the optimal load of vines with fertile buds for all varieties and cultivation forms of vines, in accordance with the technology of grape production, which will give the most favourable production results. Any subsequent engagement of labour in order to regulate and correct the yield during the growing season additionally burdens production.

Viticulture has a long tradition in Montenegro and indigenous grape varieties Vranac, Kratošija, Krstač and Žižak play a very important role in the viticulture and winemaking sector (Maraš et al., 2012). In modern viticultural production, the variety is a very important factor of success, because its unique genotype directly affects the yield of grapes, sugar content and total acids in the must as basis of economic and technological characteristics (Sefo et al., 2020).

Žižak variety is an autochthonous Montenegrin variety. Burić (1995) states that quality white refreshing wines are made from its grapes and in some years also wines of top quality. Except in the vineyards in Podgorica, individual vines of this indigenous variety can still be found in Crmnica and in Montenegrin coast (Savić, 2003). Maraš et al. (2015) state that grapes of Žižak variety are used in certain areas of Boka for the production of Prošek dessert wine.The aim of this research was to examine the influence of different load of vines with fertile buds on some biological and technological characteristics of autochthonous white wine variety Žižak. Based on the results, the optimal load of vines with buds that will enable the achievement of high quality grapes and wine is to be determined.

MATERIAL AND METHODS

The research of the influence of different vine loads with fertile buds on yield and quality of grapes of the Žižak variety was performed during 2015 and 2016. The study was performed at the experimental filed of the Biotechnical Faculty in Podgorica. The experimental vineyard was planted in 2005 with a planting distance of 2.5 m between rows and 1 m within the row. The training system of the vine is a two-arm horizontal cordon with a tree height of about 80 cm. Short and mixed pruning was applied. The experiment includes the following variants: V1 - 14 buds (2 long spurs with 5 buds each and 2 spurs with 2 buds each); V2 - 18 buds (2 canes with 7 buds each and 2 spurs with 2 buds each); V3 - 20 buds (2 canes with 8 buds each and 2 spurs with 2 buds each); V4 (control) - 24 buds (2 canes with 10 buds each and 2 spurs with 2 buds each).

The research was performed on 120 vines, that is, in three replications with 10 vines for each variant. During the two-year study, the percentage of emerged and fertile shoots, potential, relative and absolute fertility rate, grape yield (kg/vine), bunch weight (g), and sugar (%) and acid (g/l) content in must were examined.

- -The percentage of total developed shoots per vine was determined from the ratio of the number of developed shoots and the number of buds left with pruning,
- -The percentage of fertile shoots per vine was determined from the ratio of the number of fertile and the number of total developed shoots,
- -The average number of flowers per bud (potential yield rate) was obtained from the ratio of the total number of flowers and the number of buds left with pruning,
- -The average number of flowers per shoot was determined from the ratio of the total number of flowers and the number of developed shoots (relative fertility rate),
- -The average number of flowers per fertile shoot (fertility rate) was obtained from the ratio of the total number of flowers and the number of fertile shoots,
- -The yield of grapes was obtained by measuring harvested grapes, and the bunch weight was determined from the ratio of the yield from ten vines and the number of bunches.
- -The content of sugar in the most was determined hydrometrically (Oechsle hydrometer), and the content of total acids in the must by neutralization of all acids and their salts with n/10 NaOH solution using the bromothymol blue indicator.

Data from the meteorological station in Podgorica were used in the analysis of climatic conditions in Lješkopolje. Data were processed by analysis of variance for a completely randomized block design. Significance of differences was determined using the LSD test.

RESULTS AND DISCUSSION

Climate conditions

Meteorological conditions prevailing in production regions have high influence on quantity and quality of grape yield (Mirošević and Karoglan – Kontić, 2008). Of all the climatic factors, the air temperature has the most dominant influence on the growth of vine, its fertility, as well as quantity and quality of grape yield. Based on the data from Table 1, the average annual air temperature in the first year of research was 17.2 °C, which is 0.6 °C higher than in 2016. Mean temperature in the vegetation period was also higher in the first year of research and was 23.2 °C. In the studied period, the highest vegetation sum of temperature was measured in 2015 (4973.1°C)

Table 1. Average monthly, annual and vegetative air temperature (ºC)

Year	*Months*												*Year average*	*Veg. Average*	*Veg sum*
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII			
2015.	6.4	7.7	11.0	14.7	21.1	25.6	31.0	29.0	24.1	17.0	11.9	6.6	17.2	23.2	4973.1
2016.	6.5	10.8	11.5	17.2	18.6	24.7	28.3	27.6	22.1	15.9	10.4	5.3	16.6	22.0	4723.0
Average	6.4	9.2	11.2	15.9	19.8	25.1	29.6	28.3	23.1	16.4	11.1	5.9	16.9	22.6	4848.0

The results given in Table 2 show that in Podgorica vineyard area, the average annual rainfall for 2015 was 1176.0 mm, while in 2016 it was significantly higher and amounted 1993.7 mm. Observing the sum of precipitation in the vegetation period, only 438.0 l/m^2 of rain fell in 2015, which is significantly less than in 2016, when 899.5 l/m^2 of water sediments were measured during the vegetation period.

Table 2. Average monthly, annual and vegetative rainfall (mm)

Year	*Months*												*Year average*	*Veg sum*
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2015.	233.2	184.8	186.7	63.8	38.9	28.7	3.6	64.7	43.6	194.7	133.3	0.0	1176.0	438.0
2016.	240.1	273.3	316.0	82.6	268.2	158.7	78.0	3.8	84.4	223.8	264.1	0.7	1993.7	899.5
Average	236.6	229.0	251.3	73.2	153.5	93.7	40.8	34.2	64.0	209.2	198.7	0.3	1584.8	668.5

The percentage of developed shoots as an indicator of yield of the variety, depends on several factors, among which variety has high importance, ecological conditions in the production year, method of pruning, length of pruning of the fertile tree, fertilization and health of vines (Popović, 2007). Based on the data presented in Table 3, the highest percentage of developed shoots (91.17%) was recorded on the least loaded vines, that is, in vines on which short pruning was applied. Variant V2 (88.35%) had a slightly lower percentage of developed shoots, while the lowest percentage of developed shoots (85.02%) was in the variant with the highest load of vines. Žižak had a significantly higher percentage of developed shoots in all tested variants in the first year of research (89.34%) compared to 2016, when it was 86.52%.

The percentage of fertile shoots (Table 3) was also the highest in vines on which 14 and 18 buds were left, respectively, and amounted 84.25 and 84.45%. In the variant in which two canes of 10 buds each and two spurs with two buds each were left, which is the usual pruning of this variety in the experimental field in Lješkopolje, the percentage of fertile shoots was the lowest and amounted 81.95%. Observing the percentage of fertile shoots by years of research, it can be noted that in 2015 (85.3%) was significantly higher compared to the second year of research when it was 81.87%.

Table 3. Developed and fertile shoots (%)

Variant	Developed shoots (%)		Average	Fertile shoots (%)		Average
	2015	2016		2015	2016	
V1	93.75	88.60	91.17	88.20	80.30	84.25
V2	89.30	87.40	88.35	85.70	83.20	84.45
V3	88.10	86.30	87.20	84.70	82.70	83.70
V4	86.24	83.80	85.02	82.60	81.30	81.95
Average	89.34	86.52	87.93	85.30	81.87	83.58

Potential fertility rate or the number of flowers per activated bud (Table 4) was highest in variants V2 and V1, accounting 1.07 and 1.06, respectively, while in vines with a load of 20 and 24 buds, this coefficient was significantly lower and accounted 0.97 and 0.91, respectively. The influence of climatic conditions on this fertility indicator was not pronounced in studied years.

Tab.4. Potential, relative and absolute coefficient of yield of Žižak variety

Variant	Average number of inflorescences per bud		Average	Average number of inflorescences per shoot		Average	Average number of inflorescences per fertile shoot		Average
	2015	2016		2015	2016		2015	2016	
V1	1.02	1.10	1.06	1.09	1.24	1.16	1.24	1.54	1.39
V2	1.09	1.05	1.07	1.22	1.20	1.21	1.43	1.46	1.44
V3	0.99	0.96	0.97	1.13	1.11	1.12	1.33	1.34	1.33
V4	0.92	0.91	0.91	1.07	1.09	1.08	1.30	1.34	1.32
Average	1.00	1.00	1.00	1.12	1.16	1.14	1.32	1.42	1.37

The relative yield rate had the highest value (1.21) in vines on which 14 buds were left in winter pruning, while the lowest number of flowers per shoot (1.08) was measured in vines loaded with 24 buds. A higher number of flowers per shoot in all tested variants were during the first year of research, which is a consequence of more favourable climatic conditions in this year of research.

The coefficient of fertility was approximately equal in both studied years. As shown in Table 4, variants V2 (1.44) and V1 (1.39) stood out in terms of number of flowers per fertile shoot, while in variants with a load of 20 and 24 buds this yield indicator had significantly lower values - 1.33 and 1.32, respectively. Pejović (1982) stated that with the increase of the vine load with buds, the average number of flowers per bud, per shoot and fertile shoot decreases, which is in accordance with the results of this research. Savić (2003) states that the average number of flowers per bud is 0.97, 1.15 per developed shoot, and 1.38 per fertile shoot which is also in line with results of this study.

The grape yield per unit area, as an absolute indicator of the variety yield, is conditioned by a large number of factors. Among the most important of them

are the biological characteristics of the variety, production technology and ecological conditions in year of research (Cindrić et al., 2000). From the results shown in Table 5, it can be noted that the lowest yield of grapes (2.74 kg/vine) was measured in the variant V1 (14 buds), while the highest yield was achieved in the variant with the highest load of grape buds - 3.60 kg/vine. Statistical data processing showed that the yield of grapes in the variant with the lowest load of vines in the two-year average is significantly lower compared to other loads applied. Other differences found in grape yields were without statistical significance. The achieved yields in studied years were at the level stated for Žižak variety by other authors in the same agroecological conditions (Savić, 2003; Maraš et al. 2012; Popović et al. 2017). According to Burić (1995), Žižak is a variety with medium to high yields ranging from 10 to 15 t/ha.

Number of bunches - The number of bunches per vine depends on the method of pruning, ecological growing conditions, but also on the applied agricultural techniques (Cindrić et al., 2000). With the increase of the load with fertile buds, from the variant with load of 14 buds to the variant with 24 buds per vine, the number of harvested bunches significantly increased. The variant with 14 buds (14.86) had the lowest number of bunches per vine in the two-year average, while the variant V4 (22.15) had the highest number of bunches. The 24 - bud variant had significantly very higher number of bunches compared to all other tested variants. A significantly higher number of bunches was also determined by comparing variants V3 (19.55) and V2 (19.30) with variant V1.

Table 5. Bunch weight and grape yield of Žižak variety

Variant	Number of bunches			Bunch weight (g)			Yield (kg/vine)		
	2015	2016	2015-2016	2015	2016	2015-2016	2015	2016	2015-2016
V1	14.33	15.40	14.86	196	174	185	2.81	2.68	2.74
V2	19.62	18.99	19.30	186	169	178	3.65	3.21	3.43
V3	19.88	19.23	19.55	172	170	171	3.42	3.27	3.34
V4	22.24	22.00	22.12	165	159	162	3.67	3.50	3.60
Average	19.00	18.90	18.95	180	168	174	3.38	3.16	3.27

Parameter	Number of bunches		Bunch weight		Grape yield	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2015-2016	0.622	0.875	5.650	7.982	0.399	0.562

Bunch weight - The results of the study of the average bunch weight of Žižak variety are shown in Table 5. Analyzing the two-year average values, it can be noted that in vines loaded with 24 buds the bunch weight was the lowest and accounted 162 g, while the highest bunch weight was in vines with 14 buds - 185 g. In variant V1 (14 buds), the average bunch weight was significantly very higher in relation to other loads. Variants V2 -178 g and V3-171 g had also a significantly higher bunch weight compared to variant V4. All tested load variants had a higher bunch weight in the first year of the study. The higher

average bunch weight in the first year of the experiment is a consequence of significantly more favourable environmental conditions in this year, especially concerning temperature. During 2015, the average air temperatures in the vegetation period were significantly higher compared to 2016. The obtained results are in accordance with the results obtained by Popović et al. in 2013, which state that the bunch weight of the Vranac variety in the Podgorica vineyards area was also higher in climatically more favourable years. The average bunch weight in these studies was at the level of values that are stated by Maraš et al. (2012) and Popović et al. (2017) for variety Žižak in the same agroecological conditions.

Table 6. Sugar and acid content in the must of variety Žižak

Variant	Sugar content (%)			Acid content (g/l)		
	2015	2016	2015-2016	2015	2016	2015-2016
V1	22.42	21.73	22.00	5.95	5.80	5.87
V2	22.63	21.97	22.32	5.70	5.60	5.65
V3	22.13	21.33	21.70	6.20	6.00	6.10
V4	21.77	20.99	21.35	6.35	6.20	6.27
Average	22.23	21.50	21.84	6.05	5.90	5.97

Parameter	Sugar content		Acid content	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2010-2012	0.471	0.664	-	0.358

The sugar content in must, apart from the variety, degree of ripeness and health condition of the grapes, climatic conditions in the phase of grape ripening; significantly depends on the production technology (Ranković-Vasić, 2011). Based on the results shown in Table 6, it can be noted that the highest sugar content in must was measured in the variant V2 (22.32%), and the lowest in the variant with the highest load of vine with buds (21.35%). Statistical processing of the data showed that variant V2 has significantly higher sugar content in the must than the variant V4 and a significantly higher content in relation to the variant V1. There is also a significant difference between the 14-bud variant and the 24-bud variant. Similar results were obtained by Pejović (1982) and Maraš et al. (2012) who obtained higher sugar content in must in variants with a lower load of vines with fertile buds. The sugar content in must in all tested variants was higher in 2015, which is a direct consequence of the different meteorological conditions that prevailed in this year of the experiment. In 2016, with lower average vegetation air temperature and a higher amount of precipitation in the vegetation period, lower sugar content was found in must. Vukosavljevic et al. came to similar results (2011) who also found a higher sugar content in must in years with higher mean vegetation temperatures.

The acid content in the must is an important indicator of the quality of grapes, because the taste and harmony of grapes and produced wine depends on their presence (Popović, 2020). The results of two - year study demonstrated that the average acid content in must was satisfactory and characteristic for Žižak variety grown in agroecological conditions of Podgorica vineyard area. The highest content of acids in must was found in variant V4 (6.27 g/l), while with the lowest content was measured in variant V2 (5.65 g/l). Statistical processing of the data showed that the variant with 24 buds load per vine had a significantly very higher acid content in the must compared to vines loaded with 14 buds (5.87 g/l) and 18 buds - 5.65 g/l.

In vines with a load of 20 buds, the acid content was also significantly higher in relation to the V2 variant. These results are in line with the results of Popović et al. (2017), Maraš et al. (2012), Savić (2003), while Burić (1995) states higher values for the acid content in the must of Žižak (6-9 g/l), which is probably a consequence of different agroecological conditions in which their research was conducted.

CONCLUSIONS

Based on the conducted research, it can be concluded:

- The percentage of total and fertile shoots during the two-year study was highest in vines that were loaded with 14 buds, that is, in vines on which short pruning was applied, while the lowest percentage of total and fertile shoots in both examined years was the variant with the highest load of vines with fertile buds.
- The potential fertility rate was approximately the same in examined years. The highest number of inflorescences per bud had a variant with 18 buds-1.07, while the variant with the lowest fertility rate (0.91) had a variant with 24 buds. The average number of inflorescences per shoot and fertile shoot was also higher in variants with a lower load of vine. According to the number of inflorescences per fertile shoot (1.44), variant V2 stood out, in which 2 canes with seven buds and two spurs with two buds were left by pruning.
- The average grape yield in the two-year period was the highest in vines loaded with 24 buds, while the lowest yield was measured in vines loaded with 14 buds.
- The number of bunches varied significantly between the tested variants and ranged from 14.86 in variant V1 to 22.12 in variant V4.
- The average weight of bunches varied from 162 to 185 g. The highest weight of bunches had vines loaded with the lowest number of buds, that is, vines where short pruning was applied.
- The average sugar content in the must in the two-year period was the highest in the 18 buds variant. The higher sugar content in all tested variants was in 2015, which is a consequence of higher average air temperatures in the grapevine vegetation period.

- The acid content in must was characteristic of tested variety in Podgorica vineyard area. The highest acid content (6.27 g/l) was found in the variant with the highest load of vines.

REFERENCES

Burić, D. (1995): Savremeno vinogradarstvo, Nolit, Beograd.

Cindrić, P., Korać, N., Kovač, V. (2000): Sorte vinove loze, Poljoprivredni fakultet Novi Sad.

Mijatović, D., Jovanović-Cvetković, T. (2018): Zimska rezidba vinove loze, Banja Luka

Maraš, V, Košmerl, T.,Kodžulović, V., Šućur, S., Savović, A., Perišić, M. (2012): Yield and oenological potential of Montenegrin autochthonous grape varieties 'Kratošija' and 'Žižak', Journal of Hygienic Engineering and Design, 158-162

Maraš, V, Popović, T., Gazivoda, A., Raičević, J., Kodžulović, V., Mugoša, M., Šućur, S. (2015): Origin and characterization of Montenegrin grapevine varieties, Vitis 54, 135-137

Miroševic, N, Karoglan-Kontić, J. (2008): Vinogradarstvo, Agronomski fakultet, Zagreb.

Pejović Lj. (1982): Uticaj opterećenosti čokota rodnim okcima i načina održavanja zemljišta u vinogradu na neke biološke i tehnološke karakteristike sorte vranac u uslovima navodnjavanja, Doktorska disertacija, Novi Sad.

Pejović, Lj., Ulićević, M. (1992): Rezultati proučavanja nekih bijelih vinskih sorata vinove loze u okolini Titograda, Poljoprivreda i šumarstvo, Titograd.

Popović, T. (2007): Uticaj đubrenja i navodnjavanja na biološke i tehnološke osobine vinove loze sorte vranac. Doktorska disertacija. Univerzitet u Novom Sadu. Poljoprivredni fakultet.

Popović, T., Mijović, S., Pajović, R. (2013): The influence of climatic factors on the level and quality of yield of Vranac variety in Podgorica vineyards. Agriculture and forestry, Vol. 59. Issue 2: 137-145, 2013, Podgorica.

Popović, T., Kalač, A., Raičević, D., Pajović, R. (2017): Rodnost i kvalitet grožđa sorte Žižak u podgoričkom vinogorju. XXII Savetovanje o biotehnologiji, Zbornik radova 1: 231-237, Čačak, Srbija.

Popović, T., Mijović, S., Pajović-Šćepanović, R., Raičević, D. (2020): Analysis of possibilities of reducing the quantity of mineral fertilizer application using different types of organic fertilizers in Cardinal grape variety, Agriculture and forestry, Vol. 66 Issue 1: 261-268, Podgorica

Ranković–Vasić, Z., Atanacković, Z., Vujadinović, M., Vuković, A., Sivčev, B. (2011): Uticaj klimatskih faktora na kvalitet grožđa sorte Burgundac crni u vršačkom vinogorju, International Scientific Symposium of Agriculture "Agrosym Jahorina 2011", Zbornik radova, str. 177-183, Jahorina.

Vujović, D. (2013): Vinogradarstvo, Poljoprivredni fakultet Univerziteta u Beogradu, Beograd - Zemun

Vukosavljević, V., Boškov, K., Pajović, R., Stojanov, M., Đurić, M., Murtić, S., Kojović, R. (2011): Rodni potencijal i kvalitet sorte Merlo u trsteničkom vinogorju. XVI Savetovanje o Biotehnologiji sa međunarodnim učešćem, Zbornik radova, Vol. 16 (18): 385-390. Mart 2011, Čačak, Srbija.

Savić, S. (2003): Ekološki uslovi i autohtone sorte vinove loze u Crnoj Gori, Podgorica

Sefo, S., Hadžić, S., Kraljević, M., Delić, M. (2020): Uticaj različitog opterećenja čokota rodnim pupoljcima na rodnost sorte Trnjak. XXV savetovanje o biotehnologiji, Zbornik radova 1: 179-184, Čačak, Srbija.

INSTRUCTIONS TO AUTHORS

The "Agriculture and Forestry" journal publishes original scientific papers, review papers, short communications on agriculture, veterinary medicine, forestry, biology and other natural sciences. It is the endeavour of the journal to give place to papers of high scientific quality and international interest, authored by all the scientists from the South Eastern European Region, as well as other international scientist in order to stimulate contacts and exchange of knowledge fostering scientific productivity.

Manuscripts, submitted via electronic journal web system should be prepared in Microsoft Word ***(Times New Roman font, 11 pt)*** and submitted in format 17 x 24 cm ***(File / Page setup / Paper / Width = 17 cm; Height = 24 cm)***, with single line spacing ***(Format / Paragraph / Line spacing = Single)***, 2 cm margins all around ***(File / Page setup / Margins / Top = 2 cm; Bottom = 2 cm; Left = 2 cm; Right = 2 cm)***, that is approximately 44 lines per page in this format. All technical details are available in section AUTHORS / Check-list for Authors.

Manuscripts are published in English. Papers that have been published elsewhere, in whole or extracts (excerpts) of their important findings, will not be accepted. A manuscript should not exceed 10 pages. Exceptions can be made if content and quality of the paper justify it (at the discretion of the Editor).

Full research papers should include the following sections:

- Author/s name/s, Title with **DOI number**

The author's name should be placed above the title and with the author's appellation and affiliation in a footnote at the bottom of the front page. Author(s) affiliation should indicate name and address of institution, including the e-mail address of the corresponding author. Title should provide a concise but also an informative synthesis of the study (recommended not more than 100 characters including spaces). Short title (not more than 70 characters) should also be provided in order to be included in the header of the Manuscript. Ensure that the title contains the most important words that relate to the topic.

- Abstract

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions. Include the keywords and phrases you repeated in your abstract.

- Key words

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

Main text of the manuscript includes the following sections:

- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the

experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

- RESULTS followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. More details you can find in the Annex to the INSTRUCTIONS TO AUTHORS / Bibliographic style on the web page of the Journal: www.agricultforest.ac.me.

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

SUPPLY OF ARTWORK, PHOTOS: Diagrams and graphs should be provided as finished black and white line artwork or colour images. Electronic graphics included in your manuscript should be either inserted in the word document or as .gif or .jpg formats. Please check with the editor if you wish to submit any other type of graphic for conversion suitability. Photos should be supplied un-screened in original form or in electronic form. All illustration (diagrams, graphs, tables, photos) must be fully captioned. When there are a number of illustrations, the author should endeavour to reduce the amount of text to accommodate the illustrations in the limited space available for any article.

THE REVIEW PROCESS: Submitted manuscripts are reviewed anonymously by 2 referees (duble blind review): one from the journal Editorial board, one must be an international reviewer, working out of the University of Montenegro. All tracking of manuscripts and reviewers is done by the Editor. All attempts will be made to ensure submissions will be reviewed within three months after the submission. Manuscripts will be returned to the coresponding authors when each review is completed.

Examples available on **www.agricultforest.ac.me**.