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GENETIC STRUCTURE OF THREE ALBANIAN LOCAL GOAT BREEDS BASED ON MICOSATELLITE MARKERS

SUMMARY

Goats are an important livestock species in the hilly and mountainous area of Albania. The aim of the study was to estimate the genetic diversity of three local goat breeds in Albania by the use of 10 microsatellite markers. A total of 90 animals were selected as representative of three local goat breeds Velipoja, Dragobia and Smokthina. The molecular data were analyzed by different bioinformatics tools to estimate the genetic structure and genetic distances between the selected breeds. A total of 78 alleles were identified for 10 microsatellite loci. The number of alleles ranged from 4 (MAF209) to 11 (MAF70 and SRCRP5). The mean observed and expected heterozygosity values across all polymorphic loci in all populations were 0.573 and 0.67, respectively. The overall value for the polymorphic information content of all microsatellite loci was 0.637, indicating their suitability for genetic diversity analysis. The populations displayed heterozygosity deficit indicating the presence of inbreeding (F_{IS}=0.143). Global breed differentiation (F_{ST}) was 0.018, indicating a very poor genetic differentiation. The Factorial Component Analysis (FCA) and Bayesian based clustering structure analysis indicated the admixture between breeds. AMOVA indicated that only 1.89% of the variation can be explained by the differences between breeds. All breeds display a high genetic diversity, but the differentiation between them is very poor. The high level of inbreeding and admixture between breeds can be explained with the breeding practices, with the small population size and Whalund effect.

Keywords: goats, genetic diversity, microsatellites, genetic differentiation, structure analysis, heterozygosity

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INTRODUCTION

The population of goat in Albania is estimated at 774 thousand (Instat, 2020). Meat production (live weight) on 2020 was 18 thousand tones and milk production 80 thousand liters. The most frequent system of livestock management in Albania is the extensive system, which is characterized by the small-scale family farms with a small number of animals of local breeds. The main purpose of productive activities in these farms is the production of agricultural and livestock products to meet the family's food needs (Jani & Kume, 2018) . In Albania are raised mainly local goat breed. Goats are very important in the socio-economic life of community that raises them. Goats are spread out especially in hilly and mountainous area of Albania. They are resistant to diseases and very well adapted to the climate and local conditions of the region where are raised. Goat biodiversity of different populations is described using qualitative and quantitative traits (Kume *et al.,* 2012), or based on the visible profile (Bozgo *et al*., 2012). Genetic diversity of six local goat breeds is estimated previously by the use of different markers such as microsatellites (Hoda, Hyka, *et al*., 2011), AFLP (Hoda *et al*., 2012), mtDNA (Hoda *et al*., 2014) in the frame of Econogene project. The present study is focused on three local goat breeds: Dragobia, Velipoja and Smokthina. The population size of these breed is decreasing (Leka, 2019). These goat breeds are raised by the local farmers for meat and milk production. The aim of this study is to estimate genetic diversity of these local goat breeds that are not previously characterized, using microsatellite markers. Microsatellite markers due to their availability, low cost, and sufficient information content remain one of the most common markers for studies of genetic resources of farm animals (Selionova *et al*., 2021). Microsatellite markers are widely used for genetic characterization of different species such as cattle (Misrianti *et al*., 2022), water buffalo (Ünal *et al*., 2021), goat (Cañón *et al.,* 2006; Lenstra *et al*., 2017), sheep (Cinkulov *et al.,* 2008; Hoda *et al.,* 2009; Peter *et al*., 2007), carp (Biba *et al*., 2015), horse (Machmoum *et al*., 2020) *etc*.

There are many studies focused on the estimation of genetic diversity of goat breeds by the use of microsatellite markers. (Saitbekova *et al*., 1999) have estimated the genetic diversity of eight swiss goat breeds by the use of 20 bovine microsatellites. In order to analyse the genetic relationship among twelve Chinese indigenous goat breeds, twenty six microsatellite markers are used (Li *et al*., 2002). Genetic diversity and relationship between twenty Indian goat breeds were investigated based on 25 microsatellite markers (Dixit *et al*., 2012). Microsatellites are used for the genetic diversity analysis of Gohiliwari (Kumar *et al*., 2009), Ardi (Aljumaah *et al.,* 2012), Mehsana (Aggarwal *et al*., 2007), three Egyptian (Egyptian Baladi, Barki and Zaraibi) and two Italian (Maltese and Montefalcone) goat breeds (Agha *et al*., 2008), Yannan indigenous goat breed (E *et al*., 2019).

MATERIAL AND METHODS

Microsatellite genotyping

Blood samples were collected from 30 unrelated animals per each goat breed: Velipoja, Dragobia and Smokthina, according to the information provided by the farmer. Blood samples were collected with Vacutainer® system, in tubes with EDTA as anticoagulant. DNA was isolated by DNA salt out procedure (Gaaib *et al*., 2011).

A total of 10 microsatellites were used for this study and genotyped as previously described for 6 other Albanian goat breeds (Hoda, Hyka, *et al*., 2011). This panel of microsatellite loci was part of the panel used for the characterization of goat breeds from Europe and Middle East (Cañón *et al*., 2006).

Statistical analysis

The total number of alleles per locus, allelic frequencies, the private allele list, observed (HO) and expected (HE) heterozygosity for each microsatellite loci were computed using the GenAlEx 6.5 (Peakall & Smouse, 2012). Exact tests for Hardy–Weinberg equilibrium (HWE) were applied using the Markov Chain Monte Carlo method (20 batches, 5000 iterations per batch and a dememorisation number of 10,000) as implemented in GENEPOP 4.0 software (Raymond & Rousset, 1995). Polymorphic information content (PIC) was estimated for all markers in all breeds using the Cervus software (Marshall *et al*., 1998).

The Nei's gene diversity (HT), the diversity between populations (DST), the coefficient of gene differentiation (GST) values and allelic richness (AR) per each locus and population were calculated with FSTAT 2.9.4 (Goudet, 1995). The same software was used to compute F-statistics with 1000 permutations. Reynold's genetic distance, gene flow, pairwise FST values were calculated by GENETIX® software (Belkhir *et al*., 2004). STRUCTURE version 2.2 (Pritchard *et al*., 2000) was used for Bayesian clustering assignment, with 5 independent runs for each K between 2-4, applying 300000 MCMC repetitions. The most likely number of K was estimated by comparing the log-likelihood of each K-value and the estimated delta K (DK) were plotted using Evanno method (Evanno *et al*., 2005) as implemented in the Structure Harvester software (Earl & vonHoldt, 2012),. Graphic presentation of these statistics was obtained with the web-based Structure Harvester v 0.6.8 (Earl & vonHoldt, 2012). The factorial correspondence analysis (FCA) for the evaluation of the number of genetic groups was performed by GENETIX® software (Belkhir *et al*., 2004). The population assignment analysis was performed using the procedure proposed by Baudouin *et al*. (2004) as implemented in the GeneClass2 program (Piry *et al*., 2004).

A hierarchical analysis of genetic diversity using the analysis of molecular variance (AMOVA) is carried out by ARLEQUIN v 3.01.(Excoffier *et al*., 2007) in order to determine the partitioning of genetic variation between and within groups and populations. The significance levels were obtained by 1000 permutations.

RESULTS AND DISCUSSION

Genetic variation within and among breeds

The number of alleles (NA) per each locus in three goat populations are shown in Table 1. All markers were polymorphic in each population. The lowest value of expected heterozigosity were displayed by InraBern185 *at* Velipoja (0.341) and Smokthina breeds (0.388). MAF209 displayed the lowest expected heterosigosity *at* Dragobia breed (0.438), P19 is the locus that has the highest expected heterosigosity in all populations (Table 1). The FIS parameter displayed negative and positive values in different loci in each population.

Table 1. Number of alleles (NA), expected heterozygosity (H_E), allelic richness (AR) and inbreeding coefficient (F_{IS}) in each locus and population

	Velipoja				Dragobia				Smokthina			
Loci	NA	H_E	AR	F_{IS}	NA	H_E	AR	F_{IS}	NA	H_E	AR	F_{IS}
INRA023	7	0.801	6.903	0.195	9	0.792	8.863	0.032	9	0.7	8.86	-0.19
INRA063	6	0.63	5.806	-0.076	5	0.625	4.933	-0.173	5	0.645	4.867	0.225
MAF70	9	0.827	8.798	0.181	8	0.736	7.733	0.23	8	0.82	7.93	0.065
SRCRSP5	8	0.775	7.887	-0.083	10	0.833	10	0.143	10	0.688	10	0.066
ILSTS005	5	0.583	4.933	0.199	5	0.648	5	-0.102	6	0.699	5.999	0.211
P19	7	0.857	7	0.586	9	0.848	8.927	0.214	9	0.846	8.93	0.488
MAF209	3	0.444	3	0.127	4	0.438	3.933	0.01	3	0.504	3	0.074
SRCRSP7	6	0.616	5.887	0.005	6	0.72	5.93	0.259	6	0.67	5.933	0.253
ILSTS029	5	0.694	4.997	0.424*	5	0.673	4.999	0.231	5	0.657	5	0.391*
INRABER185	5	0.341	4.806	-0.039	8	0.559	7.797	-0.134	5	0.388	4.966	-0.067
*p<0.05												

Table 2. Total Number of identified alleles per locus (TNA), observed (H_O) heterozygosity, H_T (total expected heterozygosity), D_{ST} (gene diversity between populations), and G_{ST} (genetic diversity among populations), polymorphism information content (PIC), Allelic richness (AR), number of effective alleles (Ne), Shannon's information index (I).

Locus	TNA	Ho	Hs	H_T	D_{ST}	G_{ST}	PIC	AR	Ne	I
INRA023	10	0.748	0.764	0.831	0.067	0.1	0.809	9.072	5.614	1.916
INRA063	6	0.637	0.633	0.631	-0.002	-0.004	0.558	4.942	2.620	1.136
MAF70	11	0.67	0.794	0.797	0.003	0.005	0.763	8.488	4.649	1.681
SRCRSP5	11	0.732	0.765	0.771	0.006	0.009	0.739	9.292	4.241	1.708
ILSTS005	6	0.578	0.643	0.644	0.001	0.002	0.604	5.448	2.699	1.232
P19	10	0.485	0.85	0.863	0.013	0.019	0.840	9.072	6.082	1.947
MAF209	4	0.429	0.462	0.459	-0.003	-0.004	0.414	3.308	1.879	0.769
SRCRSP7	6	0.549	0.669	0.671	0.002	0.003	0.621	5.627	2.891	1.270
ILSTS029	5	0.439	0.675	0.67	-0.005	-0.007	0.614	4.924	2.817	1.219
INRABER185	9	0.467	0.429	0.431	0.002	0.002	0.409	5.932	1.796	0.898
Overall	78	0.573	0.669	0.677	0.008	0.012	0.637	6.611	3.529	1.378

A total number of 78 alleles were found for 10 loci in whole goat population (Table 2). Number of alleles ranged from 4 (MAF209) to 11 (MAF70 and SRCRSP5). In table 2 is shown genetic diversity according to Nei for each marker at the level of whole population. Observed heterozygosity (HO) ranged from 0.429 (MAF209) to 0.748 (INRA02) with a population mean value of 0.573. The values of Nei's genetic diversity range from 0.431 (INRABERN185) to 0.863

(P19) with a mean value 0.677. Diversity within populations (H_S) is 0.669 and between populations (D_{ST}) is 0.008. Meanwhile the G_{ST} value, which indicates the differentiation within breeds compared to the whole population is 0.012. This value of index indicates a very poor genetic differentiation of Albanian goat breeds, where 98.2% of genetic variation is due to the differences between individuals.

The polymorphism information content (PIC) per locus ranged from 0.409 (INRABERN) to 0.840 (P19). That means that all markers are informative except of INRA023 and INRABERN185 that have a PIC value lower than 0.5. Allelic richness varied from 3.308 (MAF209) to 9.292 (SRCRSP).

The Shannon's information index (I) ranged from 0.898 (InraBern185) to 1.947 (P19) with a mean value of 1.378. The number of effective alleles varied from 1.796 (Inrabern185) to 6.082 (P19).

In table 3 is summarized the list of private alleles by goat breeds with their respective frequency. A total of 13 private alleles were found. Only one private allele (MAF70-142) has a frequency of 5%, all other alleles have a very low frequency. In Smokthina breed only two private alleles are found.

Table 3. Summary of private alleles by goat breed

Pop	Locus	Allele	Freq
Velipoja	INRA023	201	0.017
Velipoja	MAF70	162	0.033
Velipoja	P19	172	0.017
Velipoja	INRABERN185	274	0.033
Velipoja	INRABERN185	286	0.033
Dragobia	INRA063	182	0.016
Dragobia	MAF70	164	0.016
Dragobia	MAF209	100	0.016
Dragobia	INRABERN185	266	0.017
Dragobia	INRABERN185	268	0.017
Dragobia	INRABERN185	284	0.017
Smokthina	MAF70	142	0.050
Smokthina	ILSTS005	188	0.033

The mean number of alleles (MNA) ranged from 6.1 (Velipoja) to 6.9 (Smokthina) (Table 4). Mean allelic richness (AR) ranged from 6.158 to 6.874. Each population revealed observed (H_O) and expected (H_E) heterozygosity values higher than 0.5. The values of H_E were greater than H_O in each breed, indicating the presence of inbreeding. All populations displayed heterozygosity deficit (F_{IS}), which confirms the presence of inbreeding. The highest value of F_{IS} was found in Velipoja (17.5%) and the lowest in Dragobia (8.6%). All populations have similar values of allelic richness. The mean effective number of alleles is 3.387 and Shannon index 1.374.

Table 4. Mean Number of Alleles (MNA), Number of Effective Alleles (Ne), Information Index (I), Observed (H_O), Expected (H_E) and Unbiased Expected (uHE) Heterozygosity, Fixation Index allelic (F_{IS}), Allelic richness (AR), and number of private alleles per population

Breed	MNA	Ne	I	Ho	He	uHe	F_{IS}	AR	NPA
Velipoja	6.100	3.413	1.334	0.542	0.644	0.655	0.175	6.158	5
Dragobia	6.900	3.513	1.428	0.628	0.675	0.686	0.086	6.874	6
Smokthina	6.600	3.235	1.362	0.551	0.649	0.660	0.168	6.332	2

Genetic differentiation

Heterozygosity deficit within breed (F_{IS}) ranged from -0.088 (INRABERN185) to 0.432 (P19) with a total of 0.143 for all loci (Table 5).

Table 5. Wright's F-statistics (F_{IT}, F_{IS}, F_{ST}) for each locus and all loci in three Albanian goat breeds

LocName	F_{IS}	F_{IT}	F_{ST}
ILSTS005	0.106	0.108*	0.003
ILSTS029	-0.016*	-0.011*	0.351*
INRA023	0.023	0.137	0.116
INRA063	-0.006	-0.012	-0.006
INRABER185	-0.088	-0.082	-0.006
MAF209	0.072	0.063	-0.009
MAF70	0.157*	0.161*	0.006
P19	0.432	0.444*	0.022
SRCRSP5	0.039	0.050	0.011
SRCRSP7	0.177	0.181*	0.005
Overall	0.143*	0.158*	0.018*

Table 6. Reynold's D_R genetic distance matrix (below diagonal), pairwise F_{ST} distance between breeds (above diagonal) and gene flow (Nm) (in bracket)

	Velipoja	Dragobia	Smokthina
Velipoja		0.016	0.029
Dragobia	0.017 (14.94)		0.009
Smokthina	0.03 (8.09)	0.009 (28.80)	

The highest statistically significant ($p<0.005$) contribute was provided by MAF70 (0.157). F_{IT} values ranged from -0.082 (INRABERN185) to 0.444 (P19), with a value of global heterozygosity deficit of 0.158. Genetic differentiation between breed is measured by F_{ST}. Global breed differentiation (F_{ST}) was 0.018, that is almost equal with G_{ST} value. The only marker that statistically contributed to breed differentiation was ILSTS005 (0.351) ($p<0.005$). All estimates of F statistics were significantly different from zero ($p<0.005$). This is line with the

similar frequency distribution of common allele in each microsatellite locus between populations.

Reynold's genetic distances (D_R) are shown in table 6 (below the diagonal). The smallest distance is between Velipoja and Dragobia. Pairwise F_{ST} values are very low, indicating a poor genetic differentiation between breeds. The smallest differentiation is between Dragobia and Smokthina, which also have the highest gene flow (28.80.).

In the FCA, the first Principal Component accounted for 55.8% of the variance and the second component contributed 44.2% of the variance (Figure 1). The figure 1 shows some level of admixture between breeds. This can be explained with the gene flow between breeds.

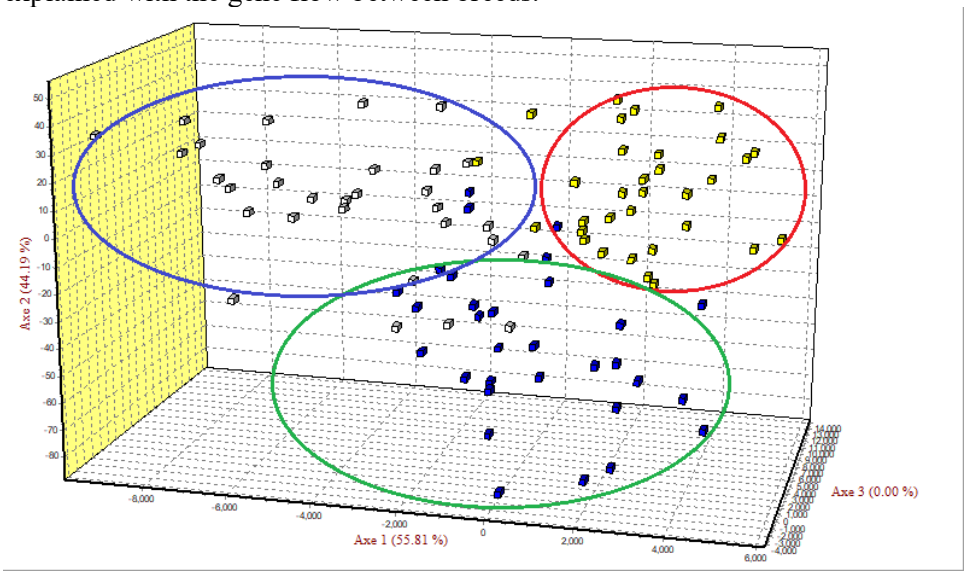

Figure 1. Factorial Correspondence Analyses (FCA) between individuals of three goat populations

In order to investigate breed differentiation, Bayesian clustering analysis was carried out as implemented by the software STRUCTURE. According to Evanno method (Evanno *et al.*, 2005), implemented in the Structure Harvester software (Earl & vonHoldt, 2012), it was assumed that k = 3 is the most likely number of ancestral populations that contribute to the genetic diversity of Albanian goat breeds (Figure 2). Results show admixture between populations, which are in line with the results of FCA.

Results of AMOVA analysis are shown in table 7 about 85.11% of variation is within individuals and variation among goat populations is 1.78%.

Population assignment analysis is performed by the use of GeneClass2 programme (Piry *et al.*, 2004), according to the procedure of (Baudouin *et al.*, 2004). The analysis indicated that 56% of individuals were correctly assigned to the population of origin, with a quality index of 42.60%.

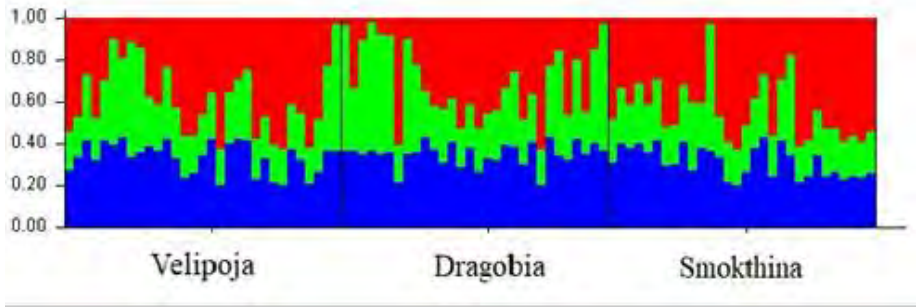

Figure 2. Genetic structure of goat breeds based on microsatellite markers under an assumption of K = 3 using the STRUCTURE software.

Table 7. AMOVA analysis results for all goat populations

Source of variation	Sum of squares	Variance components	Percentage of variation
Among populations	14.996	0.063 Va	1.89
Among individuals within populations	325.109	0.432 Vb	13.00
Within individuals	257.500	2.829 Vc	85.11
Total	597.604	3.324	

Genetic characterization of goat breeds is essential for conservation strategy as well as for genetic improvement (El-Sayed *et al*., 2017). The microsatellite markers used in this study displayed a good level of polymorphism and high values of heterozygosity. The number of alleles (Na) in a population are very important measure of genetic variability. The mean number of alleles was higher than 6 in each breed. All populations displayed similar number of alleles and number of effective alleles. MAF209 have the lowest number of alleles in each population. The average PIC value was 0.637 and only two markers have PIC values less than 0.5. Expected heterozygosity in each population is higher than 0.6 and the values of observed heterozygosity were higher than 0.5. These finding of number of alleles and the heterozygosity demonstrate high genetic diversity of the studied populations. Heterozygosity is essential for populations ant the heterozygous population is usually provides a more genotypes that are able to adapt to the harsh environment. The results indicate that the breeds had high genetic diversity. Some of the reasons for the high genetic diversity observed in a breed might be the overlapping generations, mixing of populations from different geographical locations (Serrano *et al*., 2009). The number of males used for mating is very low. The herd book are lacking and the breeding programes do not exist (Hoda *et al*., 2012). Therefore mixing of population realized by the farmers in each breed may have played role in the poor

differentiation of the goat breeds. The observed heterozygosity was less than expected heterozygosity for ach population. The deviation from Hardy Weinberg equilibrium can be explained by Wahlund effect, inbreeding, selection (Hoda, Hykaj, *et al*., 2011). Another reason of deviation from HWE might be the presence of null alleles. This is supported with the positive F_{IS} values at different marker (P19, ILSTSO) in each population. The level of inbreeding was high (14.3%). Only two loci contribute significantly to this value. Therefore in the high value of inbreeding might contribute the effect of null alleles, the Wahlund effect. Positive values of F_{IS} indicate the presence of inbreeding in each population. Dragobia displayed the lowest inbreeding level. Inbreeding is reported previously (Hoda, Hyka, *et al*., 2011; Hykaj *et al*., 2013) in other albanian goats populations. The F_{IT} indicate 15.8% general deficit of heterozygous individuals. The positive F_{IS} values are in line with the decreasing of the population size reported by (Leka, 2019).

Genetic differences between populations based on F_{ST} values are low. Pairwise F_{ST} values in all cases are lower than 0.05. Genetic differentiation might be affected by migration and random genetic drift. F_{ST} index indicated a poor differentiation between all breeds (F_{ST} = 1.8%), which is in line with the small genetic distance according to Reynold's and with the high values of gene flow. These breeds are geographically distant and the high gene flow can be explained with the breeding practices. The males are selected by the farmers without having any information about the genealogy, due to lack of herd book. The factorial correspondence analysis and structure analysis indicated admixture between breeds. The most appropriate number of clusters according to structure analysis was 3. AMOVA is applied to estimate the differentiation and genetic similarity between populations. Estimation of genetic diversity within and between populations is fundamental for designing appropriate breeding and conservation programs. AMOVA revealed that most of variation was found within individuals than among populations (1.89%). The gene flow between populations was high which might be the principal source of genetic similarity between populations (Hoda, Hykaj, *et al.,* 2011). The percentage of individuals correctly assigned to the population of origin was not very high (56%), which indicate the existence of genetic similarities between populations The microsatellite markers used in this study were sufficiently informative to estimate the genetic structure and similarity between these breeds. It is very important to maintain genetic diversity therefore breeding strategies that aim to increase effective population size and minimize genetic drift effect must be designed (Serrano *et al.,* 2009). Actually no selection schemes are applied for genetic improvement of the goat breeds. Menezes *et al.,* (2020) suggested efficient herd management for the conservation of genetic resources by avoiding breeding with related individuals, exchanging individuals among herds, and increasing the effective number, which are very appropriate also for our local goat breeds. The data provided in this study must be used for the development of breeding strategy.

CONCLUSIONS

Three local goat breeds are characterized for the first time by the use of 10 microsatellite markers. The set of markers used in this study are satisfactory informative. All goat breeds show high genetic diversity. Genetic differentiation between breeds is very poor. High level of admixture was found between breeds. The level of inbreeding is high due to small population size and Wahlund effect. The results obtained in this study must be used for the development of breeding strategy and for designing the policy how to conserve these valuable genetic resources.

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THE EFFECTS OF SEED INVIGORATING TREATMENTS ON WHEAT SEEDLING GROWTH AND ACTIVITY OF ENZYMATIC ANTIOXIDANTS

SUMMARY

Pre-sowing seed invigoration methods are promising approach to alleviate the adverse effects of abiotic stresses on seed germination and early seedling establishment. The current study was conducted to evaluate the impacts of pre-plant soaking of wheat (cv. Sardari) seeds in different concentration of salicylic acid and ascorbic acid (30, 60, 90, 120, 150, and 180 ppm) on seedling early growth and antioxidant activity. Results showed that the highest germination percentage were recorded for seed treated with dilute solutions of acid salicylic (30 and 60 ppm) and intermediate concentration of acid ascorbic (90 ppm). The mentioned concentration increased the germination rate by 80%, root length by 7 cm and shoot length by 5 cm when compared with control. Hormonal seed treatments through 60 and 90 ppm ascorbic acid induced an increased seedling dry weight. However, the highest seedling vigor was achieved by low concentration of salicylic acid. Hormonal priming with acid ascorbic had a more stimulating and increasing effect on the activity of the superoxide dismutase enzyme compared to salicylic acid. The highest activity of catalase and guaiacol peroxidase were recorded for seed primed with 90 ppm salicylic acid and 60 ppm ascorbic acid, respectively. It is concluded that hormonal seed treatments has increased seedling early growth and antioxidant activity and the best performance was related to 30 ppm salicylic acid and 60 and 90 ppm ascorbic acid. The results of the present study elucidated that hormonal seed treatments as a useful, efficient and easy invigorating approach should be seriously considered in wheat fields.

Keywords: Acid salicylic, germination rate, pre-sowing treatments, ROS scavenging, seed invigoration, seedling vigor index

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INTRODUCTION

The semi-arid regions of West Asia have special climatic characteristics and most of the rainfall occurs during winter, and this has caused the main crop of these regions to be winter wheat, barley and some cool season food legumes (Ahmed *et al*., 2022). Wheat is one of the most important crops in the world and a strategic crop in Iran. However, the most significant constraint of rainfed farming in mentioned region is the shortage of water during the planting and final stage of seed filling (Ghaffari 2010; Tavanpour and Ghaemi 2016). Most of the annual rainfall in the rainfed cold regions usually happens in autumn and winter (75% of the rains) at that time due to the severe drop in temperature, plant growth is stopped for three to four months and plant only receive 25% of the total annual rains during the active growth in spring (during the end of the tillering stage to seed maturity), this causes a significant exacerbation of drought and heat stress at the end of the growth season in many areas. Additionally, in rainfed farming, rapid and uniform emergence and swift establishment of seedlings is necessary to use the autumn rains and grow the plant sufficiently before the onset of severe cold (Arun *et al*., 2022). Specifically tolerance to temperatures below freezing is though often of pivotal importance for the survival of wheat seedling across winter and it can be affect by vernalization requirement and cold acclimation condition. Therefore, it seems that in semi-arid Mediterranean-type environments, any type of crop management that can accelerate uniform emergence can reduce autumn break, increase rainfall use efficiency and improve acclimatization to cold (Flohr *et al*., 2021). Some of the major constraints on wheat growth in semi-arid region include the timing and amount of autumn rains for sowing. In cropping systems, the autumn break is one of the most important events of any year. It is the first significant rainfall event of the winter growing season and signals the start of the growth period. On the other hand due to wide variation in the timing of the arrival of adequate autumn rains for sowing during different years and also the aggravation of this issue due to climate changes during the recent decades, it is difficult to choose a calendar date for sowing wheat seeds (Pook *et al*., 2009). Based on the previous studies, it can be speculated that seed invigoration techniques can be good crop management's option under some limiting environmental conditions *(*Hussain *et al*., 2019; Marthandan *et al*., 2020; Johnson and Puthur 2021; Cruz *et al*., 2021*).*

Seed priming is an important cost-effective method which can improve the seed germination and is widely used to synchronize and accelerate the germination of individual seeds (Hussain *et al*., 2017). Seed priming base include controlled hydration of seeds up to particular point where the preliminary and intracellular processes of germination begins; however, the radical protrusion does not take place (Singh *et al*., 2018). It is generally acknowledged that seed priming can provide improved and uniform seedling emergence by reducing the seed hydration time during the germination, increase the gene expression and also activating the pre-germinative enzymes and metabolism and also enhances the antioxidant/DNA repair activities (Forti *et al*., 2020). There are many reports on

seed priming toward improving seed germination, seedling emergence, stand establishment, crop growth, nodulation, and productivity in various cereal crop species (Khan *et al*., 2011; Tabatabaei, 2013; Hussain *et al*., 2017; Khan *et al*., 2020). Seed priming induces antioxidant enzymes activity and can significantly minimizes lipid peroxidation (Janmohammadi 2012; Khan *et al*., 2020). Despite some studies, it still retains empirical features especially in the form of on-farm priming. It has been revealed that between cost-benefit management strategies exogenous application of hormone like material minimize loss of productivity and yield under unfavorable conditions (Janmohammadi *et al*., 2012).

Use of plant growth regulators and hormones like substances as priming agents has been found to increase plant growth and significantly enhance plant mechanisms to cope with abiotic stress. Although few studies have employed hormones substances under semi-arid condition, these studies are yielding promising results (Farooq *et al*., 2013; Imran *et al*., 2013; Shah *et al*., 2019). Salicylic acid (SA) is a phenolic compound which acts as an imperative signal molecule, since it regulates metabolic and physiological processes in plant (Sharma *et al*., 2020). In addition to positive effects of increased biosynthesis of SA under some conditions, it seems that exogenous SA application can significantly improve plant abiotic stress-tolerance (Zafar *et al*., 2023). Boukari *et al*. (2019) showed that the pretreatment of alfalfa seeds with SA under salinity stress improved plant growth, water content, content of photosynthetic pigments. Also improved scavenging of reactive oxygen species (ROS) capacity through the SA application during seed pre-sowing process has been reported in the literature (Janmohammadi, 2012; Bortolin *et al*., 2020).

Ascorbic acid (ASC) performs as a co-factor for some critical enzymes and preserves the process of phytohormone intermediating signaling and numerous biological processes (Farooq *et al*., 2013). ASC also controls plant growth through affecting cell division and cell expansion, modulates plant sense, and is involved in photosynthesis, hormone biosynthesis, and regeneration of antioxidants. Shah *et al*. (2019) reported that ASC priming significantly increased chlorophyll content and grain yield in late planted winter wheat and also up-regulation of diverse enzymatic antioxidants such as super oxide dismutase, peroxidase, and catalase. However, little is known about the use of hormone like substance to improve seed germination in *semi-arid* Mediterranean-type environments with long autumn break. The aim of this study was to investigate the potential role of salicylic acid and ascorbic acid in pre-sowing seed treatment on seedling early growth and antioxidant activity of winter wheat.

MATERIAL AND METHODS

The experiments were conducted at the Research Laboratory of Crop Production, College of Agriculture, University of Maragheh, Iran. The natural deteriorated seeds (two years of storage) of winter wheat (*Triticum aestivum* L.) cultivar, Sardari, were obtained from Dryland Agricultural Research Institute (DARI). Sardari is a winter-type with mean plant height of 100 cm and is well

adapted to semi-arid region with cold winter. Sardari also is resistant to shattering is a predominant dryland landrace in Iran (Roostaei *et al*., 2018). The experiment was carried out using a completely randomized factorial design with four replicates and was developed in a petri dish system under a controlled condition of germinators. Pre-sowing treatments were including control (non-primed seeds) and hormone priming through different concentration of salicylic acid and ascorbic acid *(*30, 60, 90, 120, 150, and 180 ppm*)*. Prior to seed pre-soaking treatments, healthy, unbroken, and spotless seeds were sorted out manually, afterward; the selected seeds were surface sterilized in 0.5% sodium hypochlorite (v/v) for 10 min and washed with water to prevent the growth of microbial contaminants present on the seed surface. The excess water was removed from the seeds with absorbent paper, and the seeds were air-dried to reach the initial weight, under natural conditions. Wheat seeds were primed in the dark at 25ºC and relative humidity of 50–70% for 8 h with constant gentle shaking in a mechanical shaker. Seed germination was tested on filter papers placed in Petri dishes and moistened with sufficient distilled water. Twenty-five seeds were placed in each dish and incubated in the dark at 25 ± 1°C and germinated seed were recorded daily for nine days.

Seedling growth parameters and final germination percentage (GP) were recorded according to AOSA (1983); GP= the total seeds germinated at end of trial/number of initial seeds used 100 times. Germination rate index (GRI) calculated as = G1/1+G2/2+....+Gi/i; where G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on. The methodology of GRI followed Farooq *et al*. (2013). Seed vigor index calculated by multiplying germination (%) and seedling length on the ninth day from the beginning of germination. The RWC (fresh weight - dry weight)/ (turgid weight – dry weight) × 100 (Pieczynski *et al.,* 2013) was evaluated at the end of germination. Primed and unprimed seeds were planted under natural field condition (21 November) for assessment of mean emergence time. Mean emergence time (MET) was evaluated using $MET = \sum Dn / \sum n$, where n stands for the number of seeds that emerged on day D and D is the number of days counted from the start of emergence (Ruttanaruangboworn *et al*., 2017).

For assessment of antioxidant enzymes activity shoots of wheat seedlings were collected at the 9th day of germination, and directly placed into liquid nitrogen, then stored at -80ºC until used. Approximately 500 mg of shoot sample were homogenized in an extraction buffer containing 100 mM potassium phosphate buffer (pH 7.5), 1 mM ethylene diaminetetraacetic acid (EDTA), 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinylpolypyrrolidone (Boaretto *et al*., 2014). Superoxide dismutase (SOD) activity inhibits photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The monitoring of this inhibition is used to assay SOD activity (Giannopolitis and Ries 1977). Catalase (CAT) activity was measured in a spectrophotometer (240 nm) according to Cia *et al.* (2012), through monitoring the degradation of H2O2 at 240 nm over 1 min, in a reaction mixture containing 1 mL of 100 mM

potassium phosphate buffer (pH 7.5) and 25 µL H_2O_2 (30% solution). GPX activity (Guaiacol peroxidase) was measured by following the H_2O_2 dependent oxidation of guaiacol at 470 nm, using an extinction coefficient of 26.6 mM^{-1} cm^{-1} (Tayefi-Nasrabadi *et al*., 2011). Ascorbate peroxidase (APX) assay was determined by reaction medium containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate and 0.1 mM H_2O_2 (Alves *et al*., 2018). The activity was determined by monitoring the rate of ascorbate oxidation at 290 nm at 30 °C, and values expressed as µmol ascorbate min^{-1} mg^{-1} protein. Guaiacol peroxidase (EC 1.11.1.7) activity was measured using a reaction medium containing 50 mM phosphate buffer (pH 7), 9 mM guaiacol, and 19 mM H_2O_2 (Lin and Kao 1999). The analysis was conducted in SPSS and mean comparison was performed by LSD test.

RESULTS AND DISCUSSION

Seed priming with salicylic acid (SA) and ascorbic acid (*ASC*) had significant ($P \leq 0.05$) effects on germination percentage (GP), germination rate (GR) and seedling growth parameters of wheat as shown in Table 1.

Table 1. Comparison of means of germination and seedling growth characteristics of wheat (Triticum aestivum L.) as affected by different concentration of salicylic acid and ascorbic acid.

	GP	GR	RL	SL	SFW	SDW	RWC	MET	CAT	GPX
C	79.33^{g}	66.33^{h}	6.26^{g}	6.48^{f}	195.00^{f}	28.66^{d}	89.33^{c}	9.66^{a}	29.33^{g}	204.00^{h}
SA30	98.00^{a}	137.00^{a}	13.00^{ab}	10.43^{a}	286.33^{b}	48.00^{b}	94.00^{a}	5.33^{de}	44.66^{b}	255.33^{bc}
SA60	90.33^{bc}	100.33^{d}	10.11^{c}	9.03^{b}	225.33^{e}	41.32^{bc}	93.00^{a}	7.00^{cd}	37.66^{de}	238.00^{def}
SA90	88.00^{cd}	90.66^{f}	9.30^{cd}	8.80^{b}	233.33^{de}	38.00^{c}	90.66^{bc}	6.66^{d}	51.00^{a}	275.66^{a}
SA120	85.33^{def}	90.66^{f}	8.66^{def}	7.70^{de}	233.66^{de}	43.66^{bc}	92.00^{abc}	8.66^{b}	40.00^{bcde}	249.66^{cd}
SA150	85.66^{de}	78.66^{g}	9.56^{cd}	7.96^{cd}	231.33^{de}	42.66^{bc}	92.00^{abc}	8.00^{bc}	35.33^{ef}	231.33^{efg}
SA180	83.33^{ef}	82.33^{fg}	9.16^{cde}	7.80^{de}	206.33^{f}	34.33^{cd}	92.33^{abc}	7.66^{c}	30.33^{fg}	238.00^{def}
ASC30	83.33^{ef}	99.00^{de}	7.77^{f}	8.70^{bc}	250.33^{c}	41.66^{bc}	90.33^{bc}	5.66^{de}	44.33^{bc}	243.00^{cde}
ASC 60	96.00^{a}	100.33^{d}	11.78^{b}	10.78^{a}	288.66^{b}	57.00^{a}	92.66^{ab}	5.00^{e}	52.33^{a}	265.00^{ab}
ASC 90	92.00^{b}	90.66^{f}	13.60^{a}	10.93^{a}	313.00^{a}	65.00^{a}	94.00^{a}	5.33^{de}	45.33^{b}	273.66^{a}
ASC 120	84.33^{def}	91.00^{ef}	8.70d^{ef}	7.80^{de}	242.33^{cd}	36.66^{cd}	90.00^{bc}	6.66^{d}	40.33^{bcde}	246.33^{cd}
ASC 150	78.66^{g}	81.33^{g}	7.91^{ef}	7.02^{ef}	254.00^{c}	42.33^{bc}	91.66^{abc}	7.33^{c}	41.66^{bcd}	224.66^{fg}
ASC 180	81.66^{fg}	83.33^{fg}	8.53d^{ef}	7.64^{de}	208.33^{f}	43.66^{bc}	89.33^{c}	8.00^{bc}	39.00^{cde}	218.00^{gh}
LSD	3.79	8.07	1.29	0.826	15.85	8.55	3.42	0.36	5.33	14.70

C: No-priming (intact seeds), SA30-18: salicylic acid concentration (ppm), ASC: ascorbic acid, GP: germination percentage (%), GR: germination rate index, RL: root length of seedling (cm), SL: shoot length of seedling (cm), SFW: seedling fresh weight (mg), SDW: seedling dry weight (mg), RWC: relative water content of seedling (%), MET: mean emergence time (days), CAT: catalase activity (*Units mg $^{-1}$ protein*) and GPX*:* guaiacol peroxidase *(*Units mg $^{-1}$ protein*)*. Data with the same letter are not significantly different at p < 0.05 (*LSD:* Least Significant Difference).

However, the effect of hormone-like compounds was largely dependent on their concentrations. Assessment of GP showed that although all hormone

priming treatments were able to increase this parameter compared to the control, the highest increase was recorded in very low concentrations of SA (18%) and medium concentrations of ASC (14%). The results of the germination rate showed that despite the increase in the germination rate of primed seeds by both hormones, the rate of increase was much prominent by low concentrations of salicylic acid (30 and 60 ppm) that the germination rate was about twice as compared to the control. However, the germination rate in the seeds primed with ascorbic acid was on average about 20% higher than the no-primed seeds (Table 1). Mean comparison of the seedling root length showed that the effects of ascorbic acid and salicylic acid on this parameter were similar to each other, altogether the hormonal priming increased the root length by about 60% compared to the no-primed seeds. The longest roots were recorded in the seeds treated with 30 ppm salicylic acid or 90 ppm ascorbic acid. Affectability of shoot length from seed priming treatment was lower than root length as priming treatments only increased this component by 30% compared to the control conditions. The longest shoot recorded for seed primed with 30 and 60 ppm SA or 60 and 90 ppm ASC.

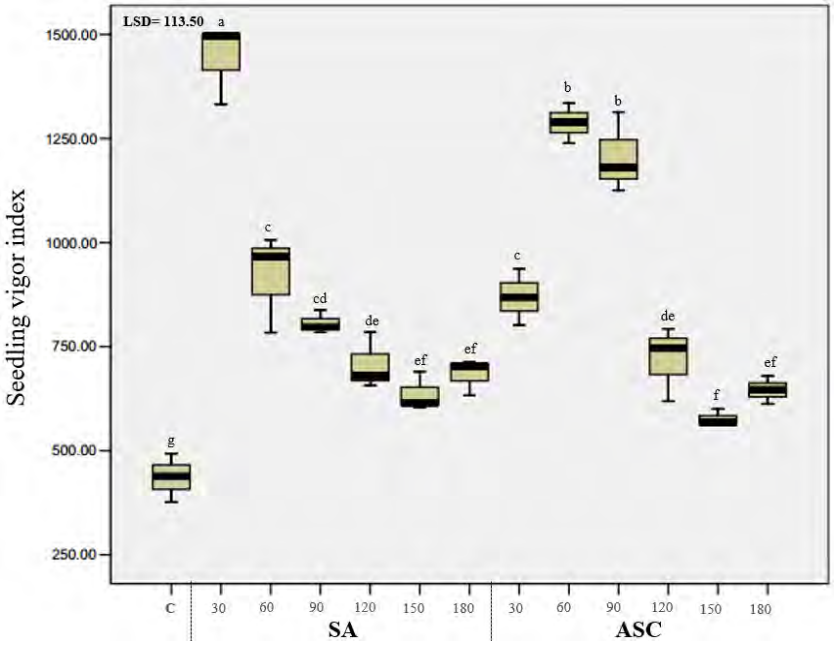

Fig. 1. Effects of wheat seeds pre-sowing treatment in different concentration of hormone-like substances on seedling vigour index. SA: C: Control or non-primed; SA: salicylic acid, ASC: ascorbic acid. All values represent means ± standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at $P \leq 0.05$ as determined by LSD test.

Assessment of seedling vigor index also showed that the highest vigor recorded for seed treated with 30 ppm of SA that followed by seeds treated with 60 and 90 ppm of ASC (Figure 1). The aforementioned priming conditions improved the vigor index by 2.5-3 times when compared with no-primed seeds. However, other priming treatments also had a positive effect on this index and increased this index by 50-75% compared to the control. The lowest positive effect of priming treatments on vigor index was observed in high concentrations of ascorbic and salicylic acid. Evaluation of seedling fresh weight showed that the heaviest seedlings were obtained from seeds primed with 90 ppm Asc. So that their weight was about one and a half times more than the weight of seedlings in intact seeds. Although both hormones used in priming increased the dry weight of seedlings compared to the control, the improving effect of ascorbic acid on seedling dry weight was more evident than salicylic acid. Seed priming with salicylic acid and ascorbic acid increased the dry weight of seedlings by 44% and 66%, respectively, compared to control. The highest dry weight of seedlings was recorded for seeds treated with 60 and 90 ppm ASC. Relative water content (RWC) of seedling affected by priming treatment ($P \leq 0.05$). The positive effect of salicylic acid on RWC was more evident than ascorbic acid and the highest RWC was recorded for seed primed with 30 and 60 ppm SA or 90 ppm ASC. Between the seedling development characteristics Mean emergence time (MET) can reflect a lot of information. MGT is a measure of the rate and time-spread of germination and emergence, focusing on the day at which most seedling have emergence.

Mean germination time decreased significantly ($p \leq 0.01$) for seedlings grown from primed seed low concentration ASC and SA. The fastest seedling emergence occurred in seeds primed with 30 and 60 ppm ASC. Seed priming with low concentration of ASC and SA decreased the MGT by 45% and 35% compared to no-primed seeds. Seed priming treatments had a significant effects on the performance of enzymatic antioxidant of wheat seedlings. Although the effect of different concentrations on the activity of antioxidant enzymes was statistically significant, these effects were somewhat different from germination indices. The highest activity of APX were recorded for seedling grown from seed primed with low concentration of SA or 120 ppm ASC (Figure 2). Other priming treatments also increased the activity of APX. However, there was a significant difference between the effects of the previously mentioned treatments and other priming treatments.

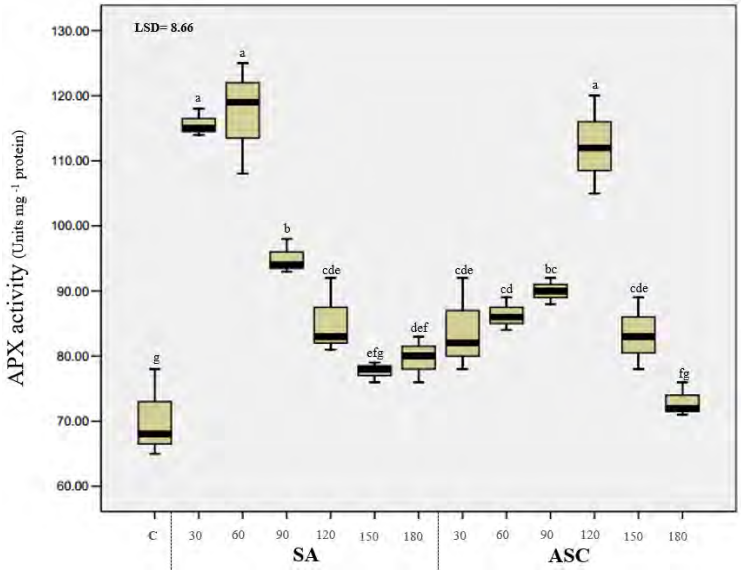

Fig. 2. The effect of hormonal seed priming on Ascorbate peroxidase activity in wheat seedling. SA: C: Control or non-primed; SA: salicylic acid, ASC: ascorbic acid. In each hormone, the numbers indicate the concentrations used for priming (ppm). All values represent means ± standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at $P \leq 0.05$ as determined by LSD test.

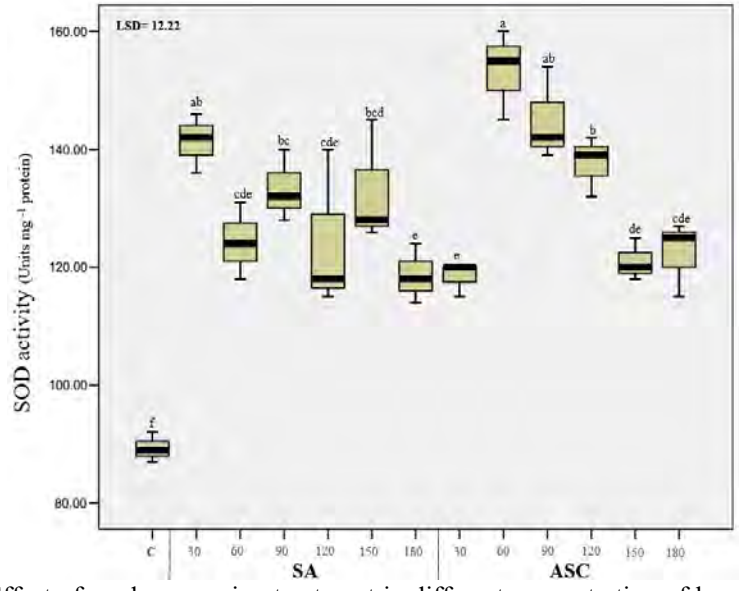

Fig. 3. Effect of seed pre-sowing treatment in different concentration of hormone like substance (ppm) on activity of superoxide dismutase in wheat seedling. SA: *salicylic acid, ASC: ascorbic acid.* All values represent means ± standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at $P \leq 0.05$ as determined by LSD test.

Furthermore evaluation of CAT activity showed that the highest level of CAT activity was related at the concentration of 120 ppm SA and 60 ppm ASC which was 75% higher than the no-primed seeds. Likewise seed priming had positive effect on activity of guaiacol peroxidase the highest GPX activity was observed in seeds treated with 90 ppm SA or Asc which was about 14% higher than the no-primed seeds. (Table 1). Assessment of SOD indicated that all priming improved the SOD activity 30-60% compared to no-primed seeds. The highest SOD activity recorded for seedling grown with seeds primed with 60 ppm ASC (Figure 3).

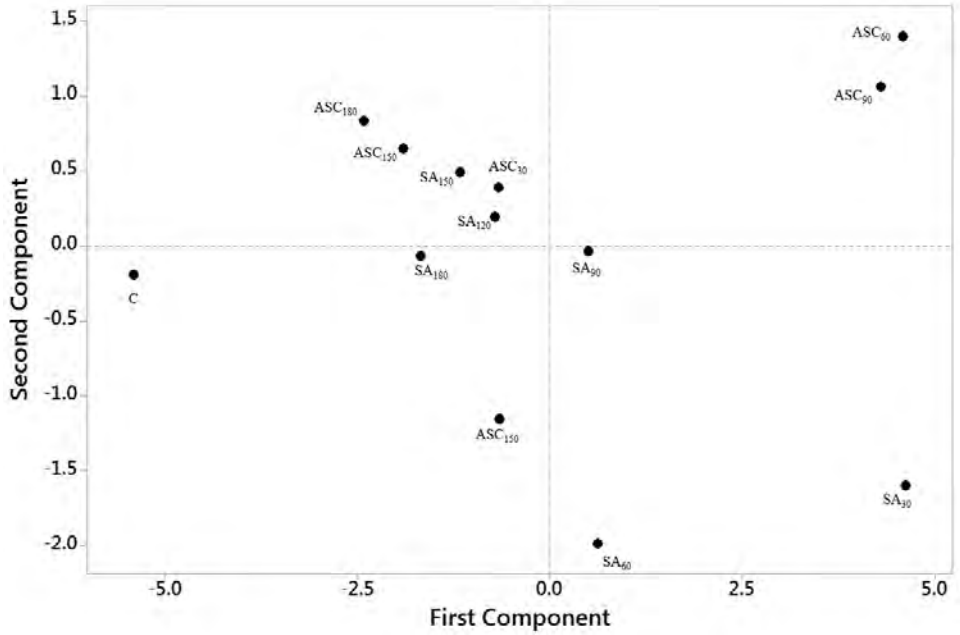

Fig. 4. Principal component analysis (PCA) based on combined treatments (hormonal substance and concentration) which measured for evaluated *seedling growth parameters*.

Figure 4 displays a biplot in the dimension of the first and second PCs. On the plot, two main groups of combined treatments were separated so that for factor 1 there were best seed priming treatment i.e. low concentration of SA and low to medium concentration of ASC. Besides, factor 2 separated control and high concentration of SA which exhibited very low performance for most of the evaluated germination parameters and activity of antioxidant enzymes. The cosine of the angle between two traits indicates their correlation situation, the smaller the angle between two traits show the more significant and positive correlation. In Figure 5, the most prominent relation is the strong positive association between germination percentage, germination rate, seedling vigor index, activity of SOD, activity of GPX and seedling length as indicated by the small obtuse angles between their vectors ($r = \cos 0 = +1$).

We were able to confirm our initial hypothesis, since pre-sowing seed treatment with hormone like substance increased the majority of studied parameters. Since the seeds were stored for more than one year in storage conditions, the obtained results showed the positive effect of hormone priming treatments on improving the germination parameters of natural deteriorated seeds. As long storage period leads to a natural deterioration of seeds and the most imperative causes for such deterioration is the increased activity of the analytical enzymes e.g. amylase, phospholipases, proteases and phytase which reduces viability and vigor of seeds (Cheyed, 2020). Considering that most of the farmers use their own seeds for planting and their storage conditions are often traditional and not favorable, hormonal priming treatments will be very important in improving the germination deteriorated seeds. The finding showed that hormone priming treatment probably improve seed germination through the increase or optimizing of the recovery process of antioxidants. Our finding showed that activity of enzymatic enzymes increased by low and medium concentration of SA and ASC. Furthermore, the used hormone-like substances themselves have antioxidant properties and can play a role in scavenging of reactive oxygen species (ROS). ASC is a small water soluble antioxidant molecule that participates as a primary substrate in the cyclic detoxification and ROS neutralization pathway, such as superoxide and singlet oxygen (Bilska *et al*., 2019). ROS levels are determined by a tightly controlled balance between production and breakdown. In general, ROS are produced unintentionally even under favorable developmental conditions (Mhamdi and Van Breusegem 2018), and if their concentration increases, they can cause the lipid peroxidation and some other injuries. In this regard, it is necessary to control the amount of ROS by increasing the activity or amount of antioxidant enzymes to survival of the seedling. This finding also corroborates the ideas of Bortolin *et al.* (2020), who suggested that seed priming with SA increased antioxidant activity of enzymes such as superoxide dismutase (SOD) and and ascorbate peroxidase (APX) in *Trifolium* species. However, our results showed that the response of antioxidant enzymes to hormone treatments is highly dependent on hormone concentrations.

Our findings indicated that seed priming with low concentration of ASC and SA resulted in vigorous, rapid, as well as uniform emergence under field condition. Average emergence time is considered as an important index in evaluating the effect of seed priming treatments on seedling strengthening in real conditions under soil physical limitations (De Ron *et al*., 2016). Seedling growth primarily relies on inherent vigor and some environmental factors such as light, temperature, water and nutrient availability. It seems that seed soaking in low concentrations of ASC and SA by activation and facilitation of germination

process can result in unique and fast growth of both root and shoot in seedling. The formerly mentioned cases accelerate the access of seedlings to environmental factors such as soil moisture, nutrients and light.

Seed soaking in low concentration of SA and low to medium concentrations of ASC gave the best growth performance of seedling, suggesting that these are ideal hormone priming for wheat under current experiment condition. The results of fresh and dry weight of seedlings indicated that although SA treatments could increase the fresh weight significantly, the highest dry weight recorded for ASC treatments. These results suggest that seedling grown from SA primed seeds had succulent growth and most of their fresh weight was based on water. This can be partly caused by osmotic regulation and better water absorption. The lowest amount of improving effect of priming treatments on germination parameters and seedling growth was observed in high concentrations of ascorbic and salicylic acid, which can be attributed to poisoning effects of high concentration or activation of other signaling pathways that were unrelated to the initiation of germination processes. The positive effects of seed priming with low concentration of hormones on germination and seedling growth can be mainly attributed to higher starch metabolism and better reserve mobilization, increased synthesis of RNA and DNA, enhanced respiration rates, higher synthesis and accumulation of metabolites, maintenance of membrane stability, and higher activities/levels of antioxidants.

CONCLUSIONS

Our results revealed that the seed priming with hormone like substance positively affect germination and seedling growth stage. However, the effect of hormones in priming conditions strongly depends on the applied concentrations. Low concentration of salicylic acid (30 and 60 ppm) and medium concentration of ascorbic acid (60 and 90 ppm) had the most positive and promising impact. Mean emergence time decreased by seed soaking in low concentration of ASC and SA under natural field condition and in late sowing date. Our results revealed that hormonal seed priming in low concentration of SA and ASC can act as a low-cost and sustainable option. In summary these priming method can be a feasible solution to solve the problems caused by autumn break in semi-arid areas, and through that, it is possible to postpone planting dates until the beginning of autumn rains. Antioxidant activity has increased in seedlings deriving from primed seeds. Results from this study suggest that a part of growth improvement can be attributed to the stimulating the activity of antioxidant enzymes, still many molecular aspects remain unknown and require further studies.

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IMPLEMENTATION OF INTERNAL CONTROL MECHANISMS AND THE POSSIBILITY OF IMPROVING FINANCIAL MANAGEMENT IN LARGE AND MEDIUM-SIZED AGRICULTURAL ENTERPRISES

SUMMARY

Countries in transition should encourage internal processes to improve management in all business segments. One of the ways to improve this is the introduction of internal control mechanisms. This way of dealing with internal factors requires a new organization, resources necessary to cover the basic and extraordinary costs of introducing internal control mechanisms, as well as additional training of employees in those entities. This paper deals with the application of three forms of internal control mechanisms, i.e. internal audit, internal control and the combination of these two forms with financial management and control. The intention was to discover the synergistic effects in large and medium-sized agricultural enterprises arising from the use of internal control factors. The obtained results are far more positive than expected, especially those related to the generation of income in terms of the size of the company and the established internal control mechanism.

Keywords: internal control, internal audit, financial management and control

INTRODUCTION

Modern management by top management in agricultural enterprises imposes a real need for improving business decision-making. Essentially, this means that there is a real need to introduce news into business decision-making processes (Popović *et al*., 2015; Bakmaz *et al*., 2017; Bjelica *et al*., 2017; Blair, 2017; Alibegović *et al*., 2018; Radović *et al*., 2019).

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Respecting the views expressed by the author, in this study the authors set as their main goal the observation of the functioning of the implementation of internal control mechanisms from the point of view of improving functional management in the operations of large and medium-sized agricultural enterprises in the Republic of Serbia, which is in accordance with already published works of the author such as (Nowak *et al*., 2016; Chen *et al.*, 2017; Murphy, 2019). In this way, the setting of the basic goal essentially meant observing the importance of applying internal control in agricultural production. In addition, the authors of the study set and carried out a research goal in this study that can be defined as emphasizing the importance of applying internal control in order to make essential improvements in the organization of agricultural enterprises, all with the aim of achieving better business results. In this way, the authors set research goals in this study with the expectation of realistically pointing out the importance of the fact that apart from the existence of the importance of applying internal control in, for example, the public sector, which was pointed out by authors such as (Wynen & Verhoest, 2016), there is also a real the need to study the application of internal control in the organization of agricultural production, which is essentially what this study draws attention to.

Based on the stated objectives of the research, the authors of the study, on the basis of the proposition they presented, which states that there is a great importance in the application of internal control in agricultural enterprises, fundamentally answered the question of whether there is an importance of the expediency of observing the application of internal control. In order to achieve this, they respected basic postulates such as the one that only management should use real business reports in order to be able to make rational business decisions, which was highlighted in works (Bakmaz *et al*., 2017; Bakmaz *et al*., 2020; Bjelica *et al.,* 2017).

The essential improvement of decision-making aims to increase the real control of the production process in all parts of the company, which is of particular importance for the organization of agricultural production, which we find in numerous works by authors (Franks & Maier, 2001; Duhovnik, 2007; Buterin *et al*., 2017; Hope & Vias, 2017; Izza, 2019; Gandi, 2019; Bojović *et al.*, 2019; Bakmaz *et al*., 2020; Vitomir *et al*., 2020). At the same time, the authors point out that they basically agreed with the stated views of the authors, but they also went a step further by observing the application of internal control from the point of view of business success in agricultural enterprises. In addition to the essential improvement of business decision-making by top management, numerous authors emphasize the importance of the application of numerous international standards, such as the application of international accounting standards, financial reporting standards, standards of the auditing profession and others, which was pointed out by numerous authors (Wang *et al*., 2014; Wang, 2016; Popović *et al*., 216; Quattrone, 2017; Popović *et al*., 2017; Toms, 2019; Radović *et al*., 2019). In addition to the implementation of standards, it is also important to consider the application of real control over decision-making

processes in companies' operations (Selarka, 2005; Williams, 2010; Majstorović *et al*., 2015; Lee, 2016; Topcu *et al*., 2017; Levis & Young, 2019. Coffee, 2019).

On the basis of the above, several important factors can be observed in business decision-making.

The first factor would be the appreciation of the application of international standards as an important factor for improving top management decision-making.

Another factor would be the application of control in order to improve real business decision-making.

The third factor would refer to the fact that top management should adopt the general postulates of business decision-making and management in companies, which they pointed out in their works (Carei *et al*., 2000; Dražić-Lutilski *et al*., 2012; Ivaniš & Popović, 2013; Jerman & Novak, 2014; Jingwen, 2017; Dmitrović-Šaponja & Suljović, 2017; Corona *et al*., 2019). The goal of such observation would be to achieve the best possible business results in the operations of companies managed by top management.

The pronounced systematized three groups of factors are indisputably important for improving business operations. They were the basis for defining the research objectives by the authors of this study. However, the authors of this study went one step further. They conducted research in which they focused on the possible increase in business safety in an agricultural enterprise. They did this by emphasizing the importance of making realistic business decisions in agricultural enterprises, especially after the introduction of the internal control system in the processes of realistic business decision-making.

MATERIAL AND METHODS

The research conducted by the authors of the study was conducted on agricultural enterprises that are registered within the Agency for Economic Registers of the Republic of Serbia (AEGRS, 2023) as enterprises that perform the main activity in agriculture. In order to enable the acquisition of correct beliefs, the authors of the study processed a sample of 54 large companies that carry out the main activity in agriculture, as well as 224 medium-sized agricultural companies in the Republic of Serbia, i.e. the study includes an analysis of 278 agricultural companies that are registered with their headquarters in the Republic of Serbia.

The author's observation and research in this study was primarily based on the observance of the Law on Accounting in the Republic of Serbia (LA-OGRS, 2019/2021) regarding the classification of companies into medium and large companies operating in the Republic of Serbia.

Based on the mentioned Law, the starting point of the authors of the study was that medium-sized agricultural enterprises in the last year of operation meet at least two of the following criteria:

- have an average of 50 to 250 employees per year,
- that they have achieved an annual income of 8,000 to 40,000 average gross earnings of employees in the Republic of Serbia,

• that the average annual property value is in the range of 6,000 to 30,000 of the average gross salary of employees in the Republic.

The application of the same Law defining large agricultural enterprises in the Republic of Serbia would be that they have more than 250 employees and that they exceed the annual income of 40,000 average gross wages of employees in the Republic of Serbia, as well as the criterion that the average annual value of assets exceeds the value of 30,000 average gross wages of employees in the Republic of Serbia.

Recognizing the stated limits on the basis of which they performed observation and research regarding the application of internal control in agricultural enterprises, the authors of the study conducted a survey in the stated number of agricultural enterprises. As part of the conducted survey, the top management was guaranteed anonymity, that is, it was guaranteed that the generalities of the company would not be presented, but that the results of the survey would be used for scientific purposes for the preparation of this study.

The next level of observation was focused on processing the data obtained by the survey. Within this part of the research, the authors set out the use of one or more forms of control in the regular operations of a large and medium-sized agricultural enterprise. According to that criterion, the observation was made in relation to the use of: internal audits, internal controls and the use of financial management and control mechanisms.

Comparing the statements of authors who basically looked at different sizes of companies, for example authors from Croatia, it is possible to see the existence of some matches with the results in this study. Namely, the authors (Mamić-Sačer *et al*., 2015) emphasized the importance of the empirical existence of the simplification of accounting regulations, because the application of such accounting could affect the reduction of the company's operating costs. This research implicitly emphasizes the importance of applying internal control in the operations of an agricultural enterprise, and indirectly confirms the importance of proper treatment of accounting by the top management that manages agricultural enterprises.

Statistical analysis

After the analysis, the authors used standard methods of statistical data processing, to then group the survey results and present them in the form of a table. After that, they performed testing using Chi-Square Tests, in order to strengthen the obtained research results.

RESULTS AND DISCUSSION

The application of different forms of internal control mechanisms was observed by the authors of this study in relation to the realized business of two types of companies of different sizes. Namely, observations were made in large and medium-sized agricultural enterprises.

Table 1. Displays the proportion of forms of application of internal control mechanisms

Form company	The values	Internal control mechanism			
		Internal Audit	Budding	Flowering	Grain ripeness
Big			26.8	41.7	9.4
			27.3	42.3	10.7
			29.1	44.4	11.3
	Count	16	29.7	47.3	11.4
	Expected Count	20.0	30.3	48.8	12.5
Medium Defective	Percentage of repre-sentation in relation to the total number of enterprises (%)	29.6	23.6	39.1	18.1
	Count	87	28.6	43.1	10.0
	Expected Count	83.0	29.3	42.4	10.9
	Percentage of repre-sentation in relation to the total number of enterprises (%)	38.8	29.7	46.7	11.2
	10		29.4	47.9	11.8
Total			0.25	1.50	0.9
	Count	103	0.85	1.60	1.10
	Expected Count	103.0	1.01	3.20	2.0

Source: Authors' calculations

The authors assumed that there is no difference in the application of the form of internal control mechanisms in large and medium-sized agricultural enterprises.

The presentation of the obtained results is given in table 1.

In addition to the above, the authors performed testing using the Chi-square test. The goal of using Chi-Square Tests was to strengthen the obtained results regarding the real business of large and medium-sized agricultural enterprises that introduced some forms of internal control in real business.

The results are shown in Table 2.

Table 2. Overview of applied forms of internal control mechanisms in enterprises using Chi-Square Tests

Used for testing	**Value**	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	11.190[a]	2	.004
Likelihood Ratio	10.486	2	.005
Linear-by-Linear Association	6.838	1	.009
N of Valid Cases	278		

Based on the obtained test results, namely the Chi-square test, it can be seen that the results are as follows. The obtained value of Pearson Chi-Square is 11.19 and the value of df is 2, where the value is $p<0.005$, which can be seen in the display in Table 2.

In the theoretical observation of the application of new forms of control in agricultural enterprises, it can be noted that top managers used the available resources that existed in the enterprises insufficiently efficiently (Moore, 1998; Nedeljković *et al*., 2022; Nožinić *et al*., 2022; Popović *et al*., 2022).

However, in this study, the authors show that the obtained results are such that the connection between the form of implemented internal control mechanisms and the business results in the mentioned two types of agricultural enterprises can be rejected with certainty (the criterion was the size of the agricultural enterprise).

The results of operations after the implementation of internal control are such that differences can be observed in the operations of medium-sized and large agricultural enterprises that have implemented internal control.

This indicates that the form of implemented internal control mechanisms is different in at least one of the analyzed groups. In addition, it can be observed that there is a significant difference in the actual application of internal control mechanisms in agricultural enterprises that differ in size.

The results obtained by the authors of this study indicate that a combination of: internal audit, internal control and financial management and control (about 46%) is most often applied in large agricultural companies. In contrast to large, medium-sized agricultural enterprises, internal control and internal audit are dominantly applied (about 38%), table 1, regardless of the size of the samples monitored by the research.

The authors of the study point out that in the Republic of Serbia, as well as in the region, there are not many works that deal with the practical study of the introduction of innovation in company management processes. Therefore, there are not many examples in the theoretical-practical sense that could indicate the possibility of real improvement of the management process through the application of internal controls. Such an application would be extremely important in the business of agricultural enterprises, because they operate with a long period of repayment of funds, face an insufficient amount of necessary funds in a certain time, and so on.

The author's research in this study was conducted in agricultural enterprises and in two important forms of agricultural enterprises. In the author's opinion, the goal of the research is satisfied because the obtained results indicate that in large and medium-sized agricultural enterprises, in addition to the need to introduce internal control, there is also a practical difference in the use of the implemented form of internal control in relation to the observed size of the agricultural enterprise. In this way, the authors determined that there are significant differences in the application of the form of internal control mechanisms, as well as that there is a connection between the size of the agricultural enterprise and the application of the form, i.e., the mechanism of using internal control in the actual work of the agricultural enterprise.

CONCLUSIONS

The results we found that there are significant differences in the application of the form of internal control mechanisms, that is, there is a connection between the size of the organization and the application of the mechanism of internal control mechanisms in the actual work of the company. We strengthened the mentioned positions after the test and it was determined that there are differences in the operations of medium-sized and large companies. There is a significant difference in the application of internal control mechanisms in companies of different sizes. In large companies, a combination of internal audit, internal control and financial management and control is most often applied (about 46%), while medium-sized companies apply internal control and internal audit to approximately the same extent (37.5 to 38.8%), while in large companies most often use a combination of internal audit, internal control and financial management and control (about 46%).

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CLIMATE CHANGE AND IMPLICATIONS ON THE ORNITHOFAUNA - CASE STUDY OF THE EURASIAN MAGPIE

SUMMARY

The aim of this study was to estimate the *Eurasian Magpie* (*Pica pica*) population and to give a detailed characterization of the nest site selection in the lower Zeta area. Data were collected in spring 2022. The findings of this study are compared to those obtained in other studies. A total of 73 active Magpie nests were censused in the River Zeta valley (lower part). One of the key factors is food availability. Breeding density, or nests, of Magpies in lower Zeta valley was 0.86 pairs/10 ha.

The results showed that the first nest building occurred at the end of February, but most pairs started in March. Nests were found in 23 trees and in two shrub species. Magpies build nests in strong and tall trees. Taller tree species were preferred to shorter ones and also broadleaved trees (ca 93%) were preferred to coniferous ones (over 40 % of all nests in 2 tree species but also over 60% of all nests in 5 tree species).The type of tree arrangement most frequently used for nesting was single trees (57.5%). The nests distance from the top of the canopy was 0.5 to 4.0 m, demonstrating the Magpie's tendency to place their nests high in the very tops of trees in the rural areas. Distance of nests from the source of food (pigsty, hen house, cowshed, stable, barn, granary, etc.) was up to 50 m in 80.8% of nests. The study shows that Magpies can adapt to changing climate factors, and changes in the choice of introduction tree species help the Eurasian magpie to adapt to climatic and anthropogenic factors.

Keywords: Magpie, *Pica pica*, nest-site selection, breeding density, nest height, rural environment.

INTRODUCTION

The Eurasian magpie is a common breeding bird widely distributed throughout the Montenegrin region. It inhabits a variety of open or semi-open habitats with hedges, bushes, or patches of trees and shrubs. In Western, Central and Southern Europe its breeding distribution is mainly associated with human-

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modified landscapes (Birkhead, 1989; Cramp & Perrins 1994; Hagemeijer & Blair 1997; Marić, 2022 a and b).

The Magpies in Europe are probably the best studied species among Corvidae and most publications refer to central and northern Europe (e.g. Witt, 1985; Jerzak, 1997, 2001; Prokop, 2004; Jokimäk *et al.* 2017), while for Montenegro a large gap in knowledge exists about range, population size and other parameters, and no data are available on breeding (area, habitat, selection of tree, etc.). Bird nests site selection is optimized by building the nest in the habitats that ensures the highest breeding success.

Several factors may drive bird nest-site selection, including predation risks, resource availability, weather conditions and interaction with other individuals. Understanding of factors where birds nest is important for conservation planning, especially in conditions of climate change, where environmental change may alter the distribution of suitable nest-sites. For the last 50 years, there have been significant changes in the nature caused by human activities, but significant variability of climate factors has also occurred. While many bird species have experienced significant declines due to expanding urbanization or abandoned areas, a number of others have adapted well to new environments and breed successfully there (e.g. Marzluff *et al*. 2001; Jerzak, 2001; Antonov & Atanasova 2003; Jokimäk *et al*. 2017; Huang, 2017; Šálek *et al*. 2021; Maric, 2022b)

More quantitative studies have addressed nest site selection in this species in general. Several studies of Magpie have shown a relationship between nest location and characteristics of nesting trees, i.e., species, their height, canopy width and etc. (Mérő *et al*. 2010; Wang *et al*. 2010; Wojciechowska & Dulicz 2014). However, there is little information on the nesting ecology of the Magpie in its area in Montenegro. Main aim was to analyze the Magpie abundance and nest site selection (nest tree species, height of nest) in Montenegro, for example the Zeta valley. This data will also reveal the assessment of some factors affecting the nesting in this study area.

MATERIAL AND METHODS

Study area

The study was carried out in the River Zeta valley (lower part, Montenegro, 42°30′N to 19°14′E, elevation 50–60 m), in the Mediterranean region. The karst depression of the Skadar basin, Zeta valley and Bjelopavlici valley, is one of the largest karst depressions in the area of the Dinarides. River Zeta is the most typical representative of karst's hydrography and, individually, the richest river in Montenegro with water, after Bojana, but with large water level oscillations. The study area characterized by a Submediterranean climate (annual temperature: 7.8 °C, precipitation: 1.200-1.500 mm) (Pešić et al. 2018 and 2020).

Along the banks of the River Zeta, there is a narrow belt (up to 20m) of trees. In some places, along the river banks, there are small forests dominated by *Quercus spp., Fraxinus spp., Carpinus betulus, Alnus spp., Ulmus minor, Salix*

spp. and *Populus spp*. *Carpinus betulus* and *Acer campestris* predominate in parts of the forest parts that have been severely altered by introduced species, such as *Ailanthus altissima*. The trees stand consists mostly of deciduous trees 100–200 years old or older. The undergrowth is very scant and the shrub layer (consisting of *Cornus mas, Rosa spp. Prunus spinosa* and *Paliurus spina-christi*, more numerous than others) covers a small area. Coniferous trees were very rare, less than 1%, mostly *Cupressus spp*. that are usually found next to human structures.

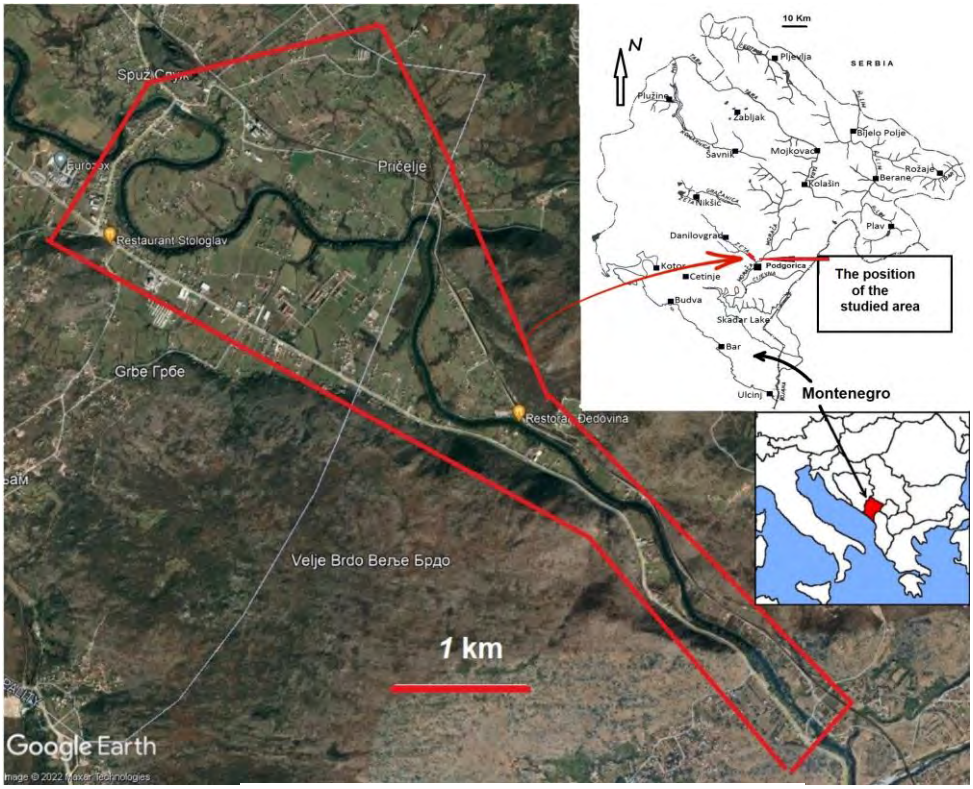

Figure 1. Study area (area bordered by red line)

The area holds one of the most representative traditional and low-intensity agricultural systems. The rural environment mainly consists of anthropogenic habitats, including built-up areas with low-density and mainly consist of green urban habitats. Green areas comprise of private gardens with a considerable proportion of non-native vegetation (evergreen shrubs and flowers). The farmland represents the area consisting of mosaic of farmed (arable fields and pastures) and non-farmed habitats (including forest fragments and non-cropped vegetation, such as hedges, isolated trees and shrub patches). In the settlement areas each private house is typically surrounded by a small garden with single trees (*Tilia spp.* oak, field elm, *Morus spp*. and mature walnut tree) and bushes. Near the houses there is often a pigsty, hen house, cowshed, stable, barn, granary,

outbuilding, garage, garden plot, orchard and lawn. The settlements are dominated by high-stem fruit orchards, and most fruit trees are mature. Fruit species are dominated by *Ficus carica*, plums, apples, pears and cherries i.e. deciduous fruit trees. Also, the trees *Robinia pseudacacia*, oak, field elm, and *Celtis australis* are found along the roads.

Data Collection

Nests were counted at the lower part of the Zeta River between Spuž and Vranske njive (Fig. 1) on a 17.7 km long road transect (measured by means of car). All Magpie nests were recorded within a range of 250 m from the road (i.e. observer) which represents an area of about 10 km2. The road, along the left and right sides of the Zeta River, borders a hill in a total length of 6.5 km, so this area (1.6 km2) was not included in the total area taken for estimation, therefore the total area calculated in this way amounts to 8.6 km2. Road transect counts of Magpie nests were performed during 12 visits in spring: from 25th February to 30th April 2022 (between 12.00 and 14.00 h), i.e. when deciduous trees were without leaves and nests could be easily seen. We systematically searched for nests on each observation point i.e. where we stopped the car. Observations were carried out with binoculars. We registered active/occupied nests, which meant that Magpies were observed in (with nest material) and around the nests (adult birds bringing fresh twigs). It is common that magpies place nesting materials in several adjacent trees before completing just one nest (Nakahara *et al*. 2015),

For each observation point we identified nest trees to the species/genus level. The tree height and the nest height placement were determined by visual method with accuracy to 0.5 m. Nest heights were measured from the ground to the bottom of the nests. Also, the following data were collected: tree species in which a nest was built, tree height and height of the nest placement in the tree, microhabitat type (single tree, tree cluster (2–3 trees, 4–10 trees or more than 10 trees), and additional descriptions of the neighboring pigsty, hen house, cowshed, stable, barn, granary, outbuilding, garages).

Statistical Analysis

The statistical analyses were performed using STATISTICA software package. All tests (t-tests, $\chi 2$-test, z test) were independent and two-tailed. Results are considered significant if $P \leq 0.05$.

Differences between nest sites in nest height (of above the ground) was tested using a t-test for independent samples. Differences between two periods in the frequency of selecting a given nest site and preference for broadleaves over conifers were tested using $\chi 2$-test.

Differences in the frequency of selecting a given nest site were tested using z test. Comparison of nest frequency in given trees between periods using z test.

A correlation-regression analysis was performed to analyze the representation of trees and the preference of nests in them.

RESULTS

Magpie (*P. pica*) nests were built between 25th February and 30th April and a new nest was built for each nesting attempt. 73 nests were observed during the present work and 24 rebuilt nests after being destroyed in storms (26 nests totally destroyed -have not been rebuilt) (see Table 1). The recorded nest density was 8.6 per km². Nests for two couples have not been found in this area. There are no statistical differences between destroyed and rebuilt nests except in the height of the selected trees. (t=2.73, p=0.009). Most of the nests (41.1%) %) were built on *Ulmus minor* and *Quercus spp.*

Table 1. Tree and bush species used as Magpie nest sites in the rural environment in Zeta river valley. RA-Relative abundance: 1) Single occurence or exceptionally rare, 2) rare , 3) average abundance, 4) abundant, 5) dominant

		First nests			Deistroyed nests			Second (rebuilt) nests		
Tree species	RA	N	(%)	Height	N	(%)	Height	N	(%)	Height
Ulmus minor	5	17	(23.3)	10-16	6	(23.1)	11-16	4	(16.8)	8-14
Quercus spp.	5	13	(17.8)	10-18	6	(23.1)	11-17	4	(16.8)	10-15
Robinia pseudacacia	4	8	(10.9)	8-12	1	(3.8)	10	1	(4.2)	10
Celtis australis	2	7	(9.6)	7-15	2	(7.7)	10-15	3	(12.5)	12-14
Fraxinus excelsior	3	4	(5.5)	10-14	-			4	(16.5)	10-12
Cupressus spp.	2	2	(2.7)	8, 12	-			1	(4.2)	8-9
Acer campestre	3	1	(1.4)	8	1	(3.8)	6	-		
Morus spp.	2	3	(4.1)	8-10	2	(7.7)	9, 10	1	(4.2)	7, 8
Pinus sp.	1	2	(2.7)	6, 10	2	(7.7)	6, 10	2	(8.3)	6, 7
Juglans regia	2	2	(2.7)	9, 10	1	(3.8)	9	-		
Malus domestica	3	2	(2.7)	6, 7	-		-	-		
Melia azedarach	1	2	(2.7)	7, 8	-		-	-		
Populus nigra	2	3	(4.1)	10-15	2	(7.7)	9, 10	-		
Populus nigra cv. italica	1	1	(1.4)	15	1	(3.8)	15	-		
Platanus sp.	1	1	(1.4)	13	1	(3.8)	13	-		
Betula pendula	1	1	(1.4)	15	-		-	-		
Broussonetia papyrifera	1	1	(1.4)	8	1	(3.8)	8	-		
Ligustrum lucidim	1	1	(1.4)	7	-		-	-		
Prunus spinosa	2	1	(1.4)	6	-		-			
Prunus cerasifera	1			-	-		-	1	(4.2)	6
Carpinus betulus	1	1	(1.4)	8	-		-	1	(4.2)	7
Ficus carica – cult.	4							1	(4.2)	5
Pirus communis	1							1	(4.2)	5
Total	23	73	(100%)		26	(100%)		25	(100%)	

For nesting, the Magpies selected common tree species rather than rare, exotic species used for gardens. Furthermore, the most preferred tree species were the *R. pseudacacia, C. australis* and *F. excelsior* (Table 1). The vast majority (94.2%) of Magpies' nests were located on trees (5.8% on shrubs) and mainly on deciduous trees (94.5%) as opposed to coniferous ones (5,5). Only one nest was located on a shrub of *P. spinosa*. There was a significant preference for broadleaves over conifers ($\chi 2 = 15.7$, df = 1, $p < 0.001$). Only two conifers (5 nests) of the 23 in total species were selected for nests. Also, only 5 nests were found in conifers of the 73 nests in total found in this area ($\chi 2 = 54.4$, df = 1, $p < 0.0001$).

Most species of fruit trees in this area were utilized to some extent by Magpies. There was a significant correlation in abundance between available trees and the number of those chosen for nesting ($r = 0.71$, $p = 0.00001$, $n = 73$).

No significant correlation in abundance between available trees and trees knocked down by wind or by the number of those chosen for nesting in second period ($p > 0.05$) (fig. 1).

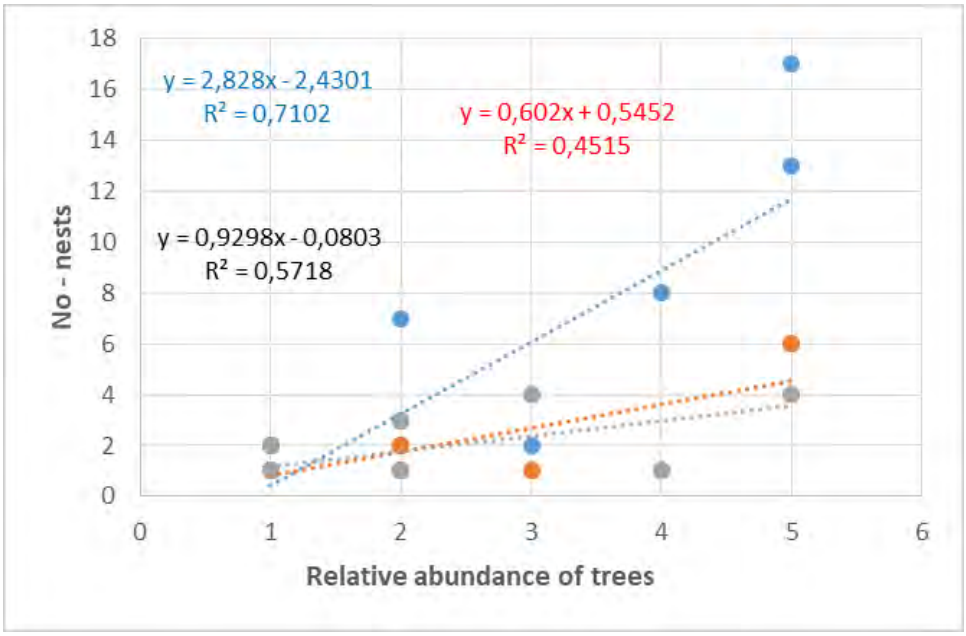

Figure 2. Correlation between relative abundance trees and number of nests (1- Single occurence or exceptionally rare, 2-rare, 3- average abundance, 4-abundant, 5- dominant)

The mean nest height was 11.85±0.14 m in the first try, whilst the mean nests height were 9.83±0.44 m in the second one. The mean destroyed nests height were 12.38±0.42 m. A significant statistical difference was found between the height of subsequently built nests (n=24) and demolished ones (n=26), as well as the mean value of all nests built (n=73) in the first period (t= 2.65, p=0,009 and t=2.73, p=0.009 respectively). No significantly differences of nests height between the first and second period of nest building (t=0.71, P=0.48).

In this study, Magpie nests were built in the upper part of the crown (canopy) and more often on the side branches than in the middle of the tree ($\chi 2=44.7$, df=1, $p < 0.0001$). Nest height was strongly correlated with nest tree height (r=0.94, $p < 0.0001$, n=73), i.e. whatever the tree height, the nest was positioned in the top part. Nests was built to the axis of tree only on four species of trees: *Cupressus spp., Pinus spp., Poplar piramidalis (Populus nigra 'Pyramidalis'*) and *Broussonetia papyrifera*. The distance between the nests varied between 20 and 450 m.

The mean distance of nests from the top of the canopy (±SD) was 2.3±0.93 m (range=0.5−4,0 m). Distance of nests from the source of food (pigsty, hen

house, cowshed, stable, barn, granary, etc.) up to 50 m was measured in 59 nests in the first and 18 in second period (80.8% and 75% respectively) and over 50 m of distance (65 to 230) was measured in 14, and 6 (19.2% and 25% respectively), so this difference is statistically significant ($\chi 2=27.7$, df=1, $p < 0.001$ and $\chi 2=6.0$, df=1, $p < 0.05$ respectively), but no significant differences between the first and second period (z=0.61, P=0.542) was noticed. Several nests were directly above the food source.

Figure 3. One example: small distance between the nests near the livestock farm.

The type of tree arrangement most frequently used for nesting was by solitary trees (57.5%) and then two clusters (2-5 and 6–10 trees) and a small groves (more than 10 trees) along the river and streams in equal proportions (see table 2).

Table 2. Number of nests by the type of tree arrangement

	Microhabitat type - single tree or tree cluster			
Tree species	single tree	2-5 trees	6-10 trees	more than 10
Ulmus minor	9 (52.9/n=17)	2	2	4
Quercus sp.	4 (30.8)	1	3	5 (38.5)
Robinia pseudacacia		2	4	1
Celtis australis	7 (100)			
Fraxinus excelsior		3	1	
Other species	22	2		1
Total	42 (57.5)	10	10	11

Figure 4. Position of the nest on the oak (*Quercus cerris*) –left, on the birch (*Betula pendula*)- right

DISCUSSION

Abundance

There is no data on magpie nesting in Montenegro. Currently, the density of the Magpie in this area is about 8.6 pairs per square kilometer, this being a small to average density compared to figures given in the consulted literature. Thus, the density of Magpie pairs observed along the riverbanks of Suhodolska and Darvenishka rivers (Bulgaria) were between 6,38 and 20,83 pairs/10ha (Kamburova, 2004). However, in a more agricultural area in Sheffield (United Kingdom), the breeding density was 0.81 pairs/10 ha (Birkhead, 1989), which is slightly lower than the densities in this area. In contrast, according to Antonov & Atanasova (2003) density in the rural habitats in Bulgaria is significantly higher and was 40.1 pairs/km2.

One of the highest known breeding densities of Magpies were inthe city Zgierz (Poland) the downtown density were 67 pairs/km2 (Wojciechowska & Dulicz 2014) then 56.8 pairs/km2, is recorded in the city of Sofia (Bulgaria) (Antonov & Atanasova 2002). However, the overall breeding density of Magpies in Sombor (Serbia) was only 0.94 pairs/10 ha (Mérő *et al*. 2010) which is significantly lower than the densities in European cities. Also, according to Bauer *et al*. (2005), the breeding densities in large cities of Central Europe vary between 0.6 and 1.4 territories/10 ha, while in smaller cities (up to 300,000 inhabitants) they do not exceed 2.1 pairs/10 ha. We conclude that specific conditions in habitats affect the number of magpies or their nests.

Trees and nests-sites characteristics

This work showed that magpies prefer certain types of trees when nesting. A total of 23 species were selected, of which 20 were selected in the first and 12 in the second nesting period, i.e. three new species, after the strong wind destroyed the first ones. Nest position or height (and the height of selected trees)

in the rebuilt new nests (24 nests) was significantly lower than in the ones that were demolished by the wind (n=26) (t=2.73, p=0.009). This and the selection of three new species shows that Magpies are very adaptable and choose nests according to the environmental and the weather conditions. Magpies selected for nesting a very wide array of tree species, which is in agreement with data from other studies. However, Vogrin (1998) found Magpie nests only in 14 woody plant species in Dravsko Polje (NE Slovenia). Considerably more types of trees have been selected in European cities: Nests were located on 47 trees and bush taxa in Zgierz and on 34 in Olsztyn (Poland) (Dulisz, 2005), on 33 tree + fruit trees (on 27 tree) in Sombor (NW Serbia) (Mérő *et al.* 2010).

The tree species preferred by Magpies in the River Zeta velley have a fairly large abundance (Table I). However, the *Ailanthus altissima* and *Salix spp*. are avoided as a nest site in this area, although they represent more abundant types of trees. It indicated that Magpies actively select (and avoid) certain species of trees in which to nest. Some study in rural habitats show that Magpies mainly place their nests in willows *Salix sp*. (59.7%) and Blackthorn bushes (Dolenec, 2000), while Vogrin (1998) found them in *Pinus silvestris* (20%) in Slovenia. These findings confirm the reported wide range of nesting tree species used by Magpies, generally according to their local abundance (Cramp & Perrins 1994). This provided many potential nesting places preferred by the Magpies.

In European cities, Manchester (UK), about 38% nests were in *Populus spp.* (Tatner, 1982), 44% nests in Zielona Góra, Poland, (Jerzak, 1995), 49% in Cracow, (Barszcz 1988) in Bonn, Germany (Witt 1985), and even 51% in Beijing,China (Wang et al. 2010). In Sombor (Serbia), magpie preferred *Celtis occidentalis* (31.1%) and *Robinia pseudacacia* (11,3) (Mérő *et al*. 2010) which is somewhat similar to our research (both species 20.5%).

The use of mainly deciduous trees for nest building in this area are in accordance with the results of researches conducted in many European countries (Jerzak, 1997., Dulisz, 2005; Wang *et al*. 2010; Tatner, 1982; Šálek *et al.* 2021). In some areas, on the contrary, according to (Jokimäki *et al.* 2017) Magpies preferred ever-green coniferous trees over deciduous tree species as their nest sites in urban areas. Also, according to Vogrin (1998) 24% of nests (4 tree species) were found on coniferous trees. In this case, the magpies in the subsequent (second) nest building period, selected a slightly different species (and a lower average height of the nest) than in the initial nest building period.
According to Kosiński (2001) or Bensouilah *et al.* (2015) this variability may be explained by the species capacity to build a nest in different nesting conditions and to adapt nest placement to the structure of available sites. Also according to Wysocki (2005), Osiński & Kempa (2007), Bensouilah *et al.* (2015), Brambilla *et al.* (2007), Şahin Aslan & Aalan Akveren (2019), etc. the birds of the same species nest at different tree species in different habitats.

The type of tree arrangement most frequently used for nesting was single trees (57.5%). This is in accordance with other studies in Europe and Asia noted by Prokop (2004); Wang *et al*. (2010); Nakahara *et al.* (2015), Huang (2017), and

Jokimäki *et al.* (2017). On the contrary, according to Xu (2020) Eurasian Magpies in Beijing (China) placed their nests preferentially in tall trees occurring in sites with high tree density, which in this research was rare, only 20%. Mérő *et al.* 2010 stated that the majority of nests were found in tree avenues (39.6%) and groups of trees (31.5%), while the lowest number of nests was recorded in solitary trees in the city of Sombor. The taller the trees were, the higher the position of the nest was from the ground. Distance of nests from the top of the canopy in this area are in accordance with the results of researches in many European countries (Jerzak, 1997., Dulisz, 2005; Wang *et al.* 2010; Tatner, 1982; Šálek *et al*. 2021). Also, this showed that Eurasian Magpies favoured tall trees as noted by many previous studies (e.g., Tucakov & Kucsera 2008; Wang *et al.* 2010; Mérő *et al*. 2010.; Nakahara *et al.* 2015, Xu *et al*. 2020., Ciebiera *et all.* 2021). However, studies carried out in rural areas e.g. in the Krapina river valley (Croatia), the nest height mean was 6.74 m, range 1−16 m (Dolenec, 2000), then, in Slovenia it was 5.7 m (Vogrin 1998), whereas in Sofia (Bulgaria) the mean nest height was 6.9 m (range 1.2−14 m) (Antonov & Atanasova 2002). This paper shows that the nests are often built on/in single trees with good all-round visibility some 6 to 18 meters off the ground, but in the most fruit trees, the height chosen was 6 or 7m. These differences probably occur due to the specific types of trees and their age. In the research area, there is a large number of species that are more than two hundred years old and reach a height of over 20 m.(e.g. *Quercus spp. Ulmus spp., Celtis sp., Fraxinus spp., Robinia sp*. *etc*). All these species, as well as *Tilia spp*. and fruit trees, are mostly present in the immediate vicinity of pigsty, hen house, cowshed, stable, barn and granary. According to our results we suggest that food availability is the main factor influencing the selection of nesting sites. According to Jerzak (2001), Kristan & Boarman (2007) and Chamberlain *et al* (2009) additional food sources such as human-provided food are important resources for some species and according to Maric (2022b) there are no Magpies in abandoned villages.

CONCLUSIONS

Magpies in the River Zeta valley nest in most available species of trees but clearly prefer some, including the *Quercus spp*. and *Ulmus minor* which is the most common species in the area. Preferred tree species typically provide a close knit canopy which (it is suggested) offers some protection to the nest site and facilitates building of the nest super-structure. In addition to *Quercus spp*. and *Ulmus minor*, most nests built in this area were in *Robinia pseudacacia* and C*eltis australis* (31%), the species which were planted very commonly in rural area. Our study shows that old *Quercus spp.* and *U. minor*, are the preferred nesting microhabitats for *P. pica*. Magpies select the best places for better breeding success. These are: proximity to food sources, visibility of micro-locations, etc. It looks as if the differential availability of human-provided food is the only factor causing the differences in densities in this area. Because the average nest height was higher in the first period than the average nest height in the second, it

suggests that birds prefer higher trees. Nest number also correlated with species tree abundance. Although there are larger numbers of willows (*S. alba*) and *Ailanthus altissima* available for nest building, the Magpie nests preferentially in large *Quercus spp*. or *U. minor*. Therefore, the maintenance of old, isolated trees in the lower section of the River Zeta is an essential condition for the conservation of this species. The present results showed that Magpies exhibited preference for certain species of tree when placing their nest, and that this is specific for the conditions in this area. Also, they showed that there was no destruction of nests in *Fraxinus excelzior* and *Cypress* (probably *Cupressus sempervirens*) and these species are recommended for planting in order to ensure nesting success in the coming period, which climatologists predict to be changeable and destructive.

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SEASONAL OCCURRENCE OF CERATITIS CAPITATA (DIPTERA: TEPHRITIDAE) ON ARGAN TREES (ARGANIA SPINOSE, SAPOTACEAE) IN MOROCCO

SUMMARY

The main objectives of this study are the assessment of *Ceratitis capitata* infestation level on Argan fruits, and monitoring of its seasonal dynamics, as well as the assessment of parasitism rate by its parasitoid *Psyttalia concolor* in Argan orchard. To study Argan fruit infestation level and the parasitism rate, infested Argan fruit were sampled and transferred immediately to laboratory. Sampled fruits were screened to determine the number of medfly punctures. Other sampled fruits were incubated in aerated plastic containers containing sterilized sand. *C. capitata* dynamics was monitored by using male attracted pheromones traps during 2018 and 2019. The obtained results show a current activity flight of *C. capitata* over the two studied years and dynamics variation between the two sampled years and between months. The peak of the *C. capitata* dynamics was recorded during May and June for 2018 and 2019 respectively. Low Argan infestation level was recorded during two periods: January and August-September. However, the peak of infestation was recorded during May (95%). *P. concolor* parasitism rate was relatively low. No significant effect of fruit origin on *P.concolor* parasitism rate ($P>0.05$) was found. Yet, the sampling period had a significant effect on parasitism rate ($p<0.05$). Argan tree is a suitable host plant

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promoting *C. capitata* development with a permanent flight dynamics year-round. Despite the mediocre and variable parasitism rate of *P. concolor*, this parasitoid can be a very important tool together with the other protective measures to reduce infestation rate of *C. capitataand* to decrease its population size.

Keywords : *Argania spinosa, Ceratitis capitata*, Infestation, Parasitism, *Psyttalia concolor*

INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann, 1924) is the most fruits fly damaging in the world. It's a polyphagous pest which infest a range wide of plants including horticultural and wild plants (Liquido *et al*., 1990; Liquido *et al.,* 1991; Morales *et al*., 2004; Copeland *et al*., 2003; Weldon *et al*., 2018). In Morocco, medfly is among major crop pests (FAOL/IAEA, 1995; Mazih, 2008). Citrus is the most important horticultural tree cultivated in Morocco with a total area of 129,000 ha and a total production of more than 2 million tons (Maroc citrus, 2022). About 50% of production is for export as fresh fruits. The current value of economic losses caused by *C. capitata* is not available. A survey carried out in 1995 in Morocco estimated the cost of loss at 53,422,200 DH based on annually losses on citrus fruits and other fruits such as Apples, Apricots, Peaches and Plums (FAO/IAEA, 1995). In addition, it is considered as quarantine pest for some countries (medfly free), such as Japan, USA, Russia and China, the exportation of fruits and vegetables to these countries requires adequate pest management in fields and cold treatment in post harvest, which raises production cost (Mazih, 2008). Host fruits of medfly in the citrus surrounding area were reported as a source of infestation (Alemany *et al.,* 2004; Martínez-Ferrer *et al.,* 2006; Ben yazid *et al*., 2020) and complicate their pest management.

Souss region is one of the most important area of citrus fruit production in Morocco. In this region, the Argan tree is the main host for medfly. Argan is an endemic tree in west-central of Morocco which covering over 821,800 ha (M'Hirit *et al.*, 1998) and playing an important socioeconomic and ecological role (Ait Aabd *et al*., 2019). The edible oil extracted from their seeds has a multiple use as food, cosmetic, and as a source of medicine. To meet the strong international demand of Argan oil, the new Argan orchards have been created. Further, a planting program of 50.000 ha was also developed and initiated as part of the new Moroccan agricultural strategy (Ait Aabd *et al*., 2022). These new established Argan orchards and Argan forest are favorable hosts for *C. capitata* which ensuring the continuity of their generations (Naamani, 2004). The Arganeraie is considered as a huge reservoir of medfly in the world (Bodenheimer, 1951; Sacantanis, 1957; Debouzie and Mazih, 1999). The ripe and ripening Argan fruits seem as a large olive fruit favorable to medfly larval development (Balachowsky, 1950; Mazih and Debouzie, 1996) which when completed is followed by pupation in the soil and then emergence of a new generation.

The main objectives of this study were the assessment of *C. capitata* infestation level on Argan fruits, the monitoring of its seasonal dynamics, and the assessment of parasitism rate of its parasitoid *Psyttalia concolor* in Moroccan Argan plantation.

MATERIAL AND METHODS

Study sites

This study has been conducted in an Argan orchard located at Belfaa (30.0434N, -9.55635W) about 50 km from Agadir city, central-west of Morocco. This Argan orchard is located in experimental farm of National Institute of Agronomic Research (INRA) in Belfaa (Figure 1). It has been planted in 2010 as an experiment of Argan breeding on a sandy soil at a density of 150 trees per hectare. The height of Argan trees is ranged from 3 to 5 meters. The drip irrigation and organic manures were applied as needed.

Figure 1. Map of studied site

Climatic data

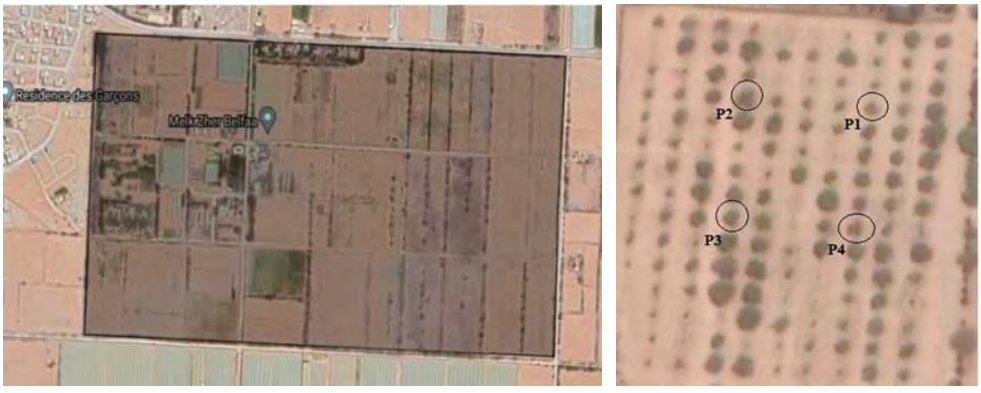
The farm is equipped by its own meteorological station, which recorded the weather parameters. Therefore, monthly temperature, rainfall and relative humidity are recorded. The Table 1 presents the average climatic data of rainfall, temperature and humidity (H) recorded monthly in Belfaa for a period of 16 years (between 1999 and 2014).

Table 1. Climatic data recorded in the studied site (Belfaa).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Tmax (°C)	21.3	22	24	24	24.6	26.4	28.5	29.1	27.7	27.3	24.4	22.3
Tmin (°C)	5.4	7.1	9.9	12	13.8	16.4	17.7	18.3	17	14.7	10.1	7.4
Tmean (°C)	13.4	14.5	16.9	18	19.2	21.4	23.1	23.7	22.3	21	17.3	14.8
Rainfall (mm)	20	25.1	26.3	11.9	4.6	0.2	0.1	1.9	4.8	13.3	22.3	28.4
Humidity (%)	63	62.9	63.1	67.5	70.1	71.7	71.9	71.2	69.8	65.6	64.2	65.4

Monitoring of *C. capitata* population

Four Argan trees were chosen for the traps installation. *C. capitata* population monitoring was designed to avoid the effect of the hedging; therefore, the four selected trees were as far away as possible from the hedge forming a square in the middle of orchard as shown in the figure 2. *C. capitata* population monitoring was performed using sticky yellow traps with a male specific pheromone (IPM Russell, UK). The traps were suspended at 1.5-2 m from the ground on the southeast side sheltered from the prevailing wind in the canopy. The pheromone was changed every four weeks and the traps were checked every two weeks during two years (2018 and 2019) to record the number of trapped males and to change the sticky yellow traps whenever necessary (De Villiers *et al*., 2013).



a **b**

Figure 2. (a) Argan orchard localization and (b) sampling design showing the four Argan trees selected for monitoring the dynamic of *C. capitata* population.

Infestation level

C. capitata infestation on Argan fruits was assessed monthly during 2018 from January to December by screening 10 fruits per tree randomly picked from the four central trees. Infested fruits were ranked according to scale adopted by Calabuig (2015) was used: 0=0 punctures; 1=1-3 punctures; 2=4-10 punctures; 3=11-30 punctures.

The infestation level was evaluated using the Townsend and Heuberger (1943) formula as showed below:

$$\boldsymbol{I}(\%) = \frac{\sum(\boldsymbol{nv})}{\boldsymbol{NV}} \times \mathbf{100}$$

n-levels of infestation according to the scale; v-number of fruits at each level of infestation; V-the total number of fruits screened; N- the highest level of the scale infestation (3 in our case)

Parasitism rate

To assess the number of *C. capitata* emerged from Argan fruits and the parasitism rate of their endoparasite P. concolor, recently fallen Argan fruits were collected every month down the four selected trees. The collected fruits were transferred immediately to the laboratory where, 10 Argan fruits per container, were placed in aerated plastic containers containing sterilized sand then incubated at temperature of 25±2°C and 70% ±5% of relative humidity (Ajerrar *et al.,* 2017). To assess the eventual difference of *C. capitata* emerged per fruits and the parasitism rate of *P. concolor* between the fallen fruits and the fruit picked from the trees, we randomly picked 10 ripe fruits in each selected trees then incubated as previously.

Data analysis

Studied parameters are the number of *C. capitata* trapped per two weeks, the mean number of *C. capitata* emerged per Argan 10 fruits and the parasitism rate of *P. concolor*. Statistical analysis was performed using Statistica software (V6, StatSoft, USA). One way and two-way ANOVA test at $p<0.05$ followed by Tukey test if significant difference was found (Ajerrar *et al*., 2020).

RESULTS AND DISCUSSION

Dynamic of Ceratitis capitate

The figure 3 shows the results of the dynamic of *C. capitata* population during the two successive years (2018 and 2019). The result shows an intra and interannual variation. Therefore, the dynamic of *C. capitata* population fluctuated between the months of the same year as well as the same months of the two sampled years. During 2018, a low dynamic between January and mid-April was registered; the mean number of males trapped during this period did not exceed 6 adults/trap days during the four surveys carried out between mid-February and mid-April. A slight increase was recorded during the second half of April where the mean number of trapped male exceeds 20 adults/trap. A significant increase of *C. capitata* dynamic was recorded during May when population reach the peak of dynamic. Therefore, more than 290 adults/trap on average was trapped. Compared to May, a slight decrease of *C. capitata* dynamic was observed during June. However, a strong decrease of the *C. capitata* dynamics was recorded from July. This decrease was progressively maintained during August and September. From the beginning of October, progressive increase of population dynamic was

shown, and consequently the mean of trapped male reached 88.5 male/trap. This increasing dynamic was maintained progressively until the end of December when the number of trapped males has exceeded 310 male/trap (Figure 3).

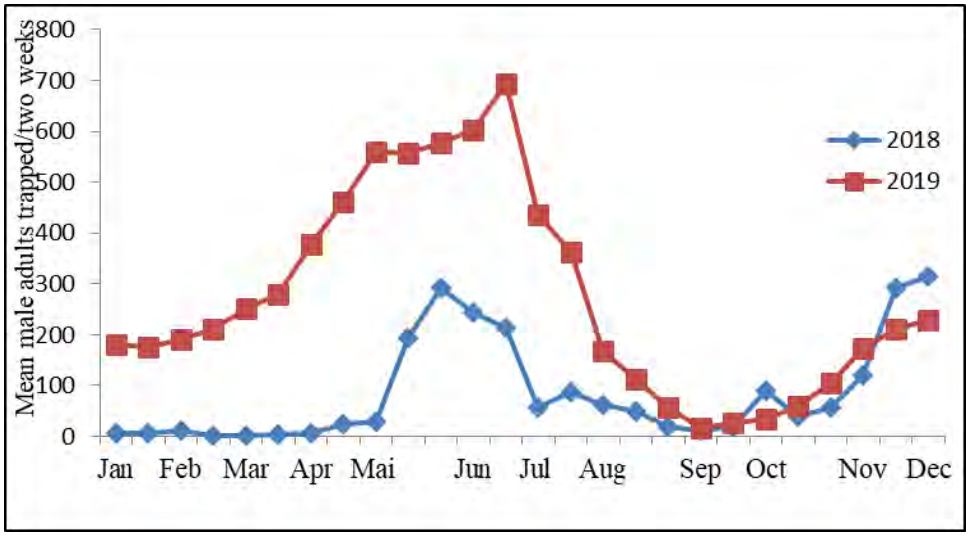


Figure 3. Population Dynamic Of *Ceratitis Capitata* During 2018 And 2019.

Compared to *C. capitata* dynamics recorded during 2018, a highly significant dynamic was recorded during 2019. Med fly dynamic was progressively increasing between January and June with a high number of male adults trapped, ranged from180 adult/trap to 280 adults/trap between January and March. After the second half of April, mean trapped male exceed 450 adult/trap and the peak of dynamic was recorded during June by 692 adult/trap. After the peak a sharp decrease was recorded during July, August and September. Therefore, 363 adult/trap was recorded in July, 57 adult/trap and 16 adult/trap during August and September respectively (Figure 3).

Infestation level of Argan fruits

A low Argan infestation level was recorded during January 2018 (7%). A progressive increase of infestation level was observed from January to March where over 50% of Argan fruits was infested; slight decrease was recorded during April (47%). Between April and May, a highly infestation level was observed; the peak of infestation was recorded during May reaching over 95% of infested fruits. A slight decrease was recorded between May and July (87%). Low infestation level was recorded during August and September which was normally characterized by the lack of Argan fruits. A sharp increase of infestation level was recorded during October (62%) with the same level during December. Significant decrease was registered during November (34%) (Figure 4).

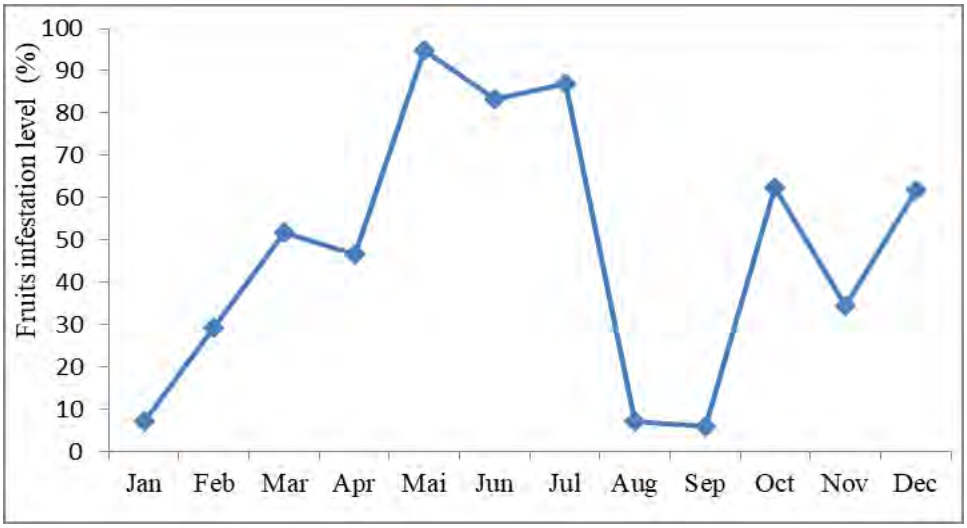


Figure 4. Argan fruits infestation level during 2018.

Results of emerged medfly per 10 Argan fruits among months during 2018 are subjected to one-way ANOVA test ($P<0.05$). No significant difference was observed on the number of emerged *C. capitata* among sampling periods (months) ($p>0.05$). Nevertheless, a high emerged medfly was observed in June 2018 with 31.7±4.44 adults per 10 fruits and a low emerged medfly was recorded during January 2018 5±6.6 (Figure 5). Also, no significant difference was recorded between the number of medfly emerged from Argan fruits collected in soil and picked ones ($p>0.05$). In addition, no significant difference was observed for interaction between sampling period and fruits origin (fallen and picked) (Table 2).

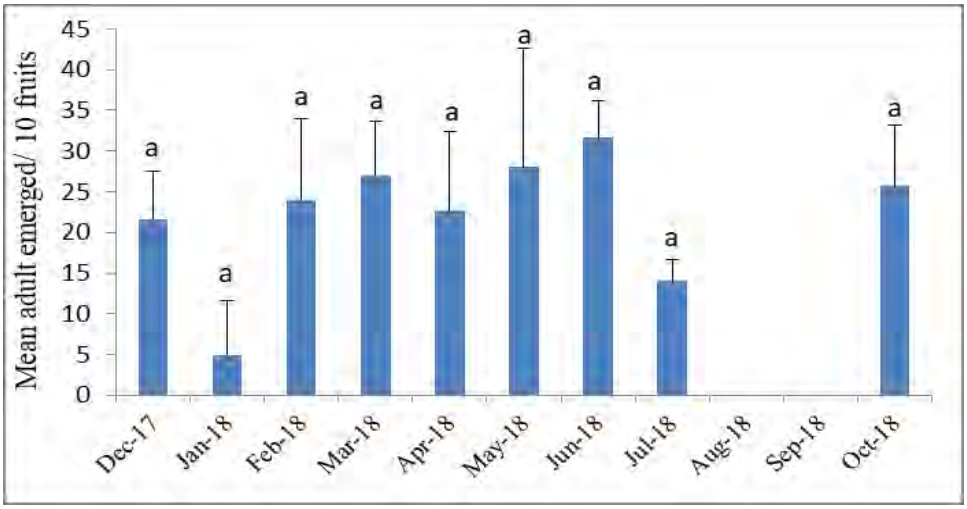


Figure 5. Mean number of *Ceratitis capitata* emerged from Argan fruits in 2018.

Table 2. Summary of ANOVA test for the effect of fruits origin (fallen /picked) and sampling period and their interaction on the number of emerged *Ceratitis capitata*.

	fd	*F*	*P*
Fruits origin	1	0.656731267	0.427254458
Sampling period	4	2.085221266	0.120781095
Interaction	4	0.176422427	0.947923237

***Psyttalia concolor* parasitism rate**

The obtained results of *Psyttalia concolor* parasitism rate are subjected to ANOVA test (P<0.05). The statistical analysis shows no significant effect of fruit origin (fallen/ picked) on parasitism rate (P>0.05). However, *Psyttalia concolor* parasitism rate shows a significant difference between the sampling periods (p<0.05). No significant impact of interaction between the fruits origin and the sampling periods on the parasitism rate was observed (P>0.05) (Table 3). A highly significant parasitism rate was recorded on fallen Argan fruit (11.85%±6.38) during May 2018. However, the low parasitism rate was reported during April and June 2018 for both fallen and picked Argan fruits with a parasitism rate less than 1% (Figure 6).

Table 3. Summary of two-way ANOVA for the effects of fruits origin (fallen /picked) and sampling periods and their interaction on parasitism rate of *P. concolor*.

	Df	*F*	*P*
Fruits origin	1	3.59424763	0.07252304
Sampling period	4	3.78994685	0.01883952
Interaction	4	0.93515119	0.46372775

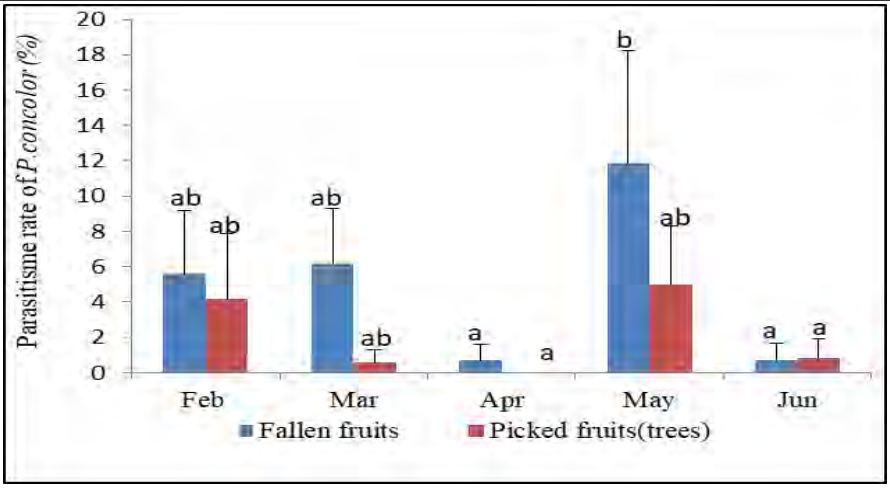


Figure 6. Parasitism rate of *P. concolor* recorded during different sampling periods. Means (±SE) with different letters indicate significant difference at P<0.05.

The monitoring of *C. capitata* dynamic allows showing the permanent activity of this pest during the whole year. This permanent dynamic can be explained by a perfect adaptation of *C. capitata* to the Argan fruits. In addition, many authors had considered Argan fruits as a perfect host of med fly (Sacantanis, 1957; Debouzie and Mazih, 1999). The results of med fly monitoring reveal a difference of mean adults trapped between the two studied years and between the different periods (months) of the same studied year. These differences may be explained by host fruits availability among the different periods. The fruiting process of Argan trees was not homogeneous during the two studied years, which leads to the time lag of availability of receptive fruits. During 2018, low dynamic was observed during two periods: between January and mid-April when receptive fruits were almost absent in the field and during August-September where receptive fruits were not available. The autumn fruiting during 2018 allows the emergence of a new generation of *C. capitata*, which in turn increased the population size during the following year. The relatively high number of trapped males between November 2018 and March 2019 can be explained by the availability of receptive fruits on some Argan trees during this period. The peak of *C. capitata* dynamics was recorded in May 2018 and June 2019. These two periods generally coincided with the peak of fruit ripening of most Argan trees in this orchard. Our results show that the number of *C. capitata* emerged per fruits was not impacted either by Argan fruits origin (fallen or picked) or by the sampling period. Moreover, no significant effect of fruits origin on *P. concolor* parasitism rate was registered. However, sampling periods had a significant effect on *P. concolor* parasitism rate. Therefore, the parasitism rate differed between periods with a high significant parasitism rate recorded during May 11.85%±6.38% and the lowest during April and June with less than 1%. This parasitism rate variation may relate with temperature difference among studied periods. Moreover, *P. concolor* development is very sensible to temperature fluctuation. According to Loni (1997), *P. concolor* development ranged between 15°C and 30°C, and no adult emergence was obtained at 13°C and 33°C. In addition, the same author state that optimal emergence rate was obtained at temperature interval ranged from 18°C to 25°C. Pearson correlation test showed that no significant relationship between the number of *C. capitata* emerged per fruit, the trapped adults (dynamic), the infestation level and the parasitism rate of *P. concolor* and the three climatic parameters recorded in Belfaa monthly (Figure 7). However, a significant relationship was observed between the infestation level and the *C. capitata* dynamics ($P<0.05$) (Figure 7). This pattern may be explained by *C. capitata* high tolerance. According to Nyamukondiwa and Terblanche (2009) *C. capitata* showed a greater thermal tolerance.

Therefore, climatic parameters recoded in Belfaa were suitable for *C. capitata* development. Moreover, the mean temperature recorded in Belfaa during the coldest month (January) and the hottest month (August) were 13.4°C and 23.7°C respectively. These values are far than critical thermal limits recorded for

C. capitata established by Nyamukondiwa and Terblanche (2009) which ranged from (5.4–6.6°C) to (42.4–43.0°C) as critical minimal and maximal temperature respectively. In addition, De Villiers *et al* (2013) study states that *C. capitata* may also be better adapted to arid climates.

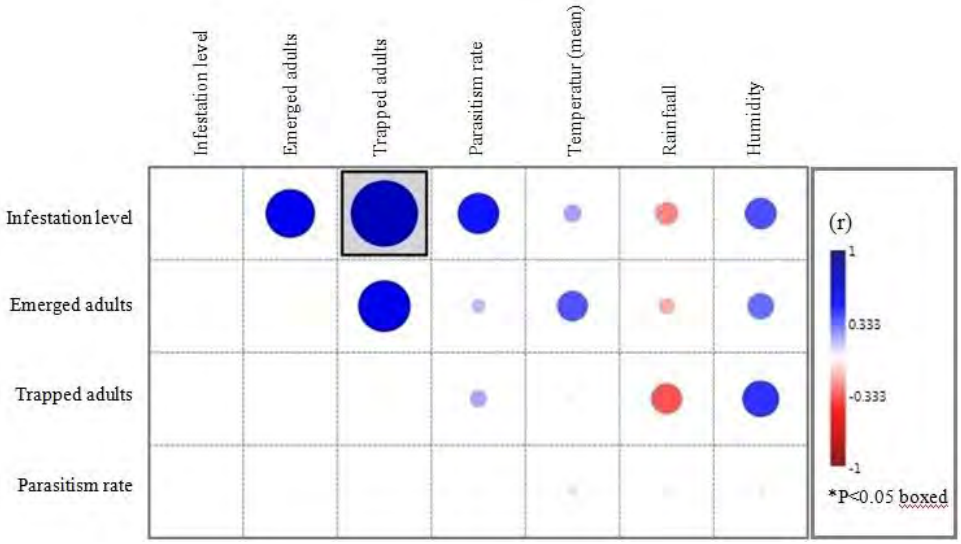


Figure 7.Summary of correlation test (Pearson) between studied parameters and the mean of climaticrecorded in Belfaa.

CONCLUSIONS

C. capitata occurrence and their flight dynamics in this Argan orchard are almost permanent. Low occurrence was recorded during a few months, mainly related to lack of ripe and ripening Argan fruits; but once the Argan fruits reach ripeness, a rapid increase in the population size was shown. This permanent occurrence is mainly explained by the favorable climatic conditions in Belfaa region, and presence of *C. capitata* in the neighboring orchards areas. Among these host plants the prickly pear and the different varieties of orange, whose ripeness period ranged from September to July, can replace the Argan fruits and thus allowing the relay of medfly generations. On the other hand, despite the mediocre and unstable parasitism rate of *P. concolor*, this parasitoid can be an important ally to other protective measures to reduce the rate of *C. capitata* infestation and to decrease the size of its population.

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ANTIOXIDANT CAPACITY AND COMPOSITION OF ESSENTIAL OIL OF LAVENDER AND LAVANDIN GROWING IN BOSNIA AND HERZEGOVINA

SUMMARY

The Lavandula genus is one of the world's most popular medicinal and aromatic plants and is rich in essential oil. Lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula intermedia* Emeric ex Loisel.) have high commercial values worldwide. However, their quality depends on genetic properties, environmental conditions, and cultural practices. Therefore, this research aimed to determine the antioxidant capacity and content of constituents in the essential oil of lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina (B&H). Samples of lavender and lavandin (cv. grosso) were collected at Butmir, B&H. The tested properties were total phenolic, total flavonoids, antioxidant activity, essential oil content and content of constituents in essential oil. The quality of tested plants was statistically significantly dependent on the cultivar. The highest values of total phenolic, flavonoids, and antioxidant capacity were recorded in the lavender (54.26 mg GAE g^{-1}, 41.49 mg CAE g^{-1} and 17.49 μM Fe^{2+} g^{-1}, respectively), while the lowest was in the lavandin (39.30 mg GAE g^{-1}, 24.07 mg CAE g^{-1} and 10.84 μM Fe^{2+} g^{-1}, respectively). Essential oil content ranged from 4.44 (Lavender) to 8.25 mL 100 g^{-1} (lavandin). Essential oil of lavender and lavandin were rich in linalool.

Keywords: lavender, lavandin, antioxidant capacity, essential oil, GC-MS

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INTRODUCTION

Since ancient times, people have been fascinated with medicinal and herbal plants because of their beneficial effects on human health. Lavender is one of the world's most popular medicinal and aromatic plants. Although there are 39 different species in this genus Lavandula, only three species have high commercial values, including Lavender (*Lavandula angustifolia* Mill.), lavandin (*Lavandula intermedia* Emeric ex Loisel.) and spike lavender (*Lavandula latifolia* Medik.) (Sönmez *et al*., 2018). Therefore, the cultivation of these species as therapeutic and aromatic herbs has increased significantly over the past few years (Kıvrak, 2018). Currently, the most famous countries for cultivating Lavender are Bulgaria, France, Italy, Spain, Turkey, Croatia, Greece, Kashmir, South Africa, and regions in Northern Africa (Vijulie *et al*., 2022).

Lavender is primarily cultivated for its essential oil, which has multiple uses. Lavender essential oil is highly valued in cosmetics, food preservation, agroindustry, traditional medicine, etc (Białoń *et al*., 2019; Mambrí *et al*., 2018; Wells *et al*., 2018; Komnenić *et al*., 2020). Lavender is used in traditional medicine for the treatment of various diseases (Behmanesh *et al*., 2015; Cardia *et al*., 2018; Firoozeei *et al*., 2021; Koulivand *et al.,* 2013; Lis-Balchin *et al*., 1999; Pandur *et al*., 2021; Puškárová *et al*., 2017). The medicinal properties of all medicinal plants, including lavender, depending on the content of phenolic compounds and their antioxidant capacity (Gavrić *et al*., 2023). Although genetic processes control the amount of these components in the plant, environmental factors and cultivation method also significantly impact their contents (Gavrić *et al*., 2018a; Albergaria *et al*., 2020; Mohammadi *et al.*, 2021; Lebedev *et al.*, 2022). However, it is known that all cultivars accumulate phenolic compounds, which are characterized by high antioxidant activity (Wells *et al*., 2018).

The quality of lavender strongly depends on the content of the essential oil and its composition of constituents. Lavender oil can have more than 100 components, most of which are terpene compounds (Sałata *et al*., 2020). Previous research (Białoń *et al*., 2019; Mambrí *et al*., 2018; Minji Hong, 2022; Sałata *et al*., 2020) has shown that lavender and lavandin essential oil contains: linalyl acetate, linalool, and γ-cadinene, p-cymen-8-ol, borneol, lavandulol, o-cymene, bornyl acetate, (E)-caryophyllene, eucalyptol, camphor. However, according to many researchers (Białoń *et al*., 2019; Erhatić *et al*., 2020; Kvaternjak *et al*., 2020; Wells *et al*., 2018), the concentration of these components in essential oils varies greatly depending on cultivars and environmental factors. For example, lavender essential oil contains 17.8-50.0% linalool, 10.1-54.0% linalyl acetate, and traces of camphor, while lavandin essential oil contains 22.5-35.5% linalool, 23.6-35.4% linalyl acetate and in 6.3-12.2% camphor (Lesage-Meessen *et al.,* 2015). On the other hand, it was found that the essential oil composition depends on the growing area's weather conditions. For example, the content of linalool, the main compound of lavender oil, increases with the increase in average daily temperature, while its content is reduced during the rainy season (Hassiotis *et al.,* 2014).

Many studies in the literature study the composition of essential oils and the antioxidant capacity of lavender, but there are no studies in Bosnia and Herzegovina (B&H). Therefore, this research aimed to determine the antioxidant capacity and content of essential oil constituents of lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina (B&H).

MATERIAL AND METHODS

Plant material and sampling

Lavender (*Lavandula angustifolia*) and lavandin (*Lavandula intermedia cv.* grosso) were used in this study. Samples were collected at Butmir, B&H (Experimental field of Sarajevo Faculty of Agriculture and Food Sciences, 43°49'34.42" N, 18°19'18.48" E; 505 m a.s.l.).

Materials for analysis

The total phenol content, total flavonoid content, antioxidant activity, and essential oil contents is determined in inflorescence samples. Samples were collected at the full flowering stage (July 19, 2021). The samples were dried in a dim environment for 30 days at room temperature. After that, collecting samples were dried and then ground to a size of 1 mm.

Extract preparation

Briefly, 1 g of each ground sample was added to a 100 mL volumetric flask, and the flask volume was filled with 60% ethanol and mixed. Then the extracts were filtered and stored in the refrigerator before the measurements.

Determination of total phenols content

The total phenolic contents of ethanolic extracts of lavender and lavandin were estimated using the Folin Ciocalteau reagent as described by Bystrická *et al*. (2011). Results were expressed as mg gallic acid equivalents per gram (mg GAE g^{-1}).

Determination of total flavonoid content

The flavonoid content of lavender and lavandin ethanolic extracts was determined using spectrophotometric methods based on forming aluminum-flavonoid complexes (Gavrić *et al*., 2018b). Results were expressed as mg catechin per gram (mg CAE g^{-1}).

Determination of antioxidant activity

The total antioxidant capacity of the extract was measured using the FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Results was expressed as μM Fe^{2+} g^{-1} of the dry sample.

Extraction and determination content of essential oils

Extracting essential oils from flower samples was carried out according to the protocol previously described by Clevenger (1928). Briefly, the dried flower samples were weighed (50 g) and then transferred to a 1000 mL volumetric flask with 500 mL of distilled water. The samples were distilled for two hours. The volume of essential oil was measured after distillation. After that, the essential oils were dried with sodium sulfate anhydrous, packed in dark vials, and stored in the refrigerator until analysis.

Gas Chromatography/Mass Spectrometry Analysis

The compositions of extracts were quantified by means of gas chromatograph with autosampler (Agilent Technologies, 7820A) equipped with capillary column (Agilent HP5-ms ultra-inert, 30 m×320 μm ×0.25 μm) coupled to an Agilent Technologies Mass Selective Detector (MSD-5977E). Temperature program used: 60 °C (1 min hold) to 246 °C (0 min hold) at a rate of 3 °C/min, and then to 280 °C at a rate of 10 °C/min. Injection volume of 1 μL was used in splitless injection mode at 220 °C and pressure of 8.4599 psi with carrier gas He of 99.9995 % purity.

Statistical methods

All experiments were performed in triplicate. One-way ANOVA was used to examine the data at a significance level of 0.05. The research findings were statistically analyzed using the SPSS 22 software.

RESULTS AND DISCUSSION

In this research, the amounts of total phenolic, flavonoids, and antioxidant capacity of the extracts from the inflorescence are shown in table 1.

Table 1**.** Effect of cultivars on total phenolic contents, total flavonoid contents, antioxidant capacity, and essential oil content

Cultivar	Total phenolics	Total flavonoids	Antioxidant capacity	Essential oil contents
	mg GAE g^{-1}	mg CAE g^{-1}	μM Fe^{2+} g^{-1}	mL 100 g^{-1}
Lavander	54.26a	41.49a	17.49	4.44c
Lavandin cv. grosso	39.30b	24.07b	10.84	8.25a
Average	**46.78**	**32.78**	**14.16**	**6.34**
Different letters indicate significant differences at the 0.05 level; ns: nonsignificant differences. GAE - gallic acid equivalent, CAE - cathetin acid equivalent.				

The findings show that the level of these bioactive components and the antioxidant capacity depended on the cultivar. The highest values of total phenolic, flavonoids, and antioxidant capacity were recorded in the lavender (54.26 mg GAE g^{-1},41.49 mg CAE g^{-1} and 17.49 μM $Fe^{2+}g^{-1}$, respectively), while the lowest was in the lavandin (39.30 mg GAE g^{-1}, 24.06 mg CAE g^{-1} and 10.84 μM $Fe^{2+}g^{-1}$, respectively). The observed differences between lavender and lavandin in the level of total phenolics can probably be attributed to genetic variations of the plant (Gavrić *et al*., 2023). Our results align with Bajalan *et al*. (2016), which also researched flavonoid content in 30 different lavender cultivars. Their research showed that the content of flavonoids ranged from 28.19 to 71.62 mg GAE CAE g^{-1} dry matter. Furthermore, the authors emphasize that it is crucial to identify lavender cultivars with a high content of flavonoids due to their positive pharmacological effects on human health. Furthermore, our results indicate that the highest antioxidant capacity was found in lavender than lavandin. Therefore, this suggests that the antioxidant capacity significantly

depends on the cultivars. In the meantime, Dobroš *et al*. (2022) studied three cultivars of lavender and two cultivars of lavandin.

They found that the cultivar greatly influences the antioxidative capacity activity. In addition to cultivars, environmental conditions such as temperature, humidity, altitude, location, and agronomic practices can significantly impact antioxidant capacity (Gavrić *et al*., 2018a).

The quality of lavender and lavandin mainly depends on the essential oil yield and its content of constituents (Sałata *et al*., 2020). Our research showed that the studied cultivars have a relatively high essential oil content. Also, analysis of essential oil content in our research showed a significant difference between the tested cultivars. The highest essential oil content was recorded in the lavandin (8.25 mL 100 g^{-1}), followed by the *Lavanda cultivars* (4.44 mL 100 g^{-1}). According to Mkaddem Mounira *et al.* (2022) variations in essential oil content can result from genetic factors, developmental stages, environmental conditions, harvesting methods, methods of drying, extraction, and analysis. Given that in our research, all factors that can influence the oil content were uniform, except for the cultivar, the resulting differences can be attributed to researched cultivars. This opinion can be supported by the finding of Renaud *et al.* (2001), who found that the essential oil content varies between 2.8-5.0% in lavender and 7.1-9.9% in lavandin.

Another important property that determines the quality and market price of essential oil of lavender and lavandin is the content of constituents. A data from the chromatogram (Figure 1 and 2) show that 23 different ingredients were isolated in essential oil of lavender and lavandin, and their percentage content is presented in Table 2.

The total of 23 ingredients were identified in the isolated essential oil, which makes up 98.06-98.30%. Furthermore, it is important to note that lavender and lavandin essential oils were different. In essential oil of lavender main isolated constituents were linalool (64.47%), β-cis-ocimene (6.50%), terpinen-4-ol (4.77%), linalyl acetate (4.22%), β-myrcene (3.38%), and other compounds in a smaller proportion. The main constituents in the essential oil of *Lavandin cv.* grosso were linalool (35.40%), eucalyptol (13.16%), camphor (9.54%), linalyl acetate (6.58%), β-myrcene (6.05%), lavandulol acetate (4.63%), β-cis-ocimene (4.56%), and other compounds.

Overall, our research determined that linalool is the main constituent of the essential oils of both cultivars. Therefore, by comparing this constituent between cultivars (Table 2), it can be said that lavender's highest proportion was recorded (64.47%). In contrast, lower linalool content was recorded in lavandin (35.40%). Our results are consistent with Lesage-Meessen *et al*. (2015), who also found higher linalool content in lavander than in lavandin cultivars.

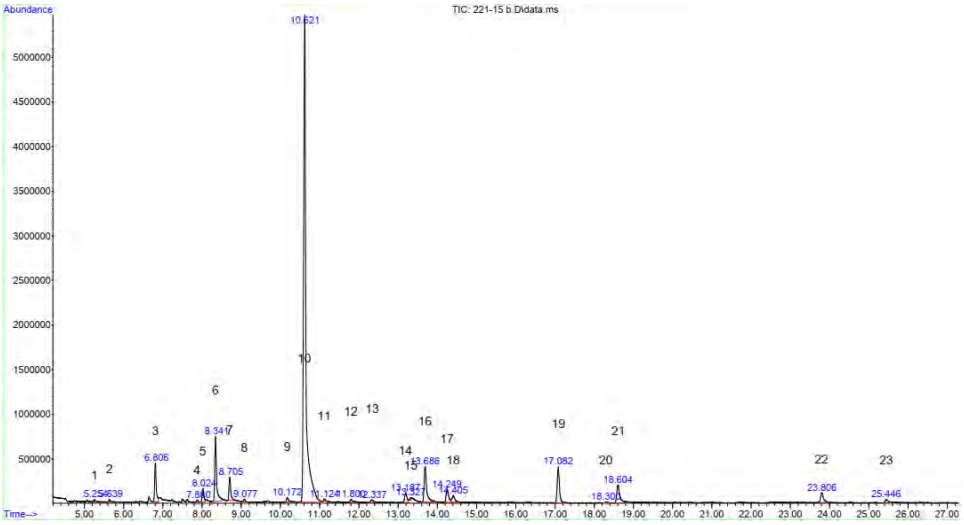

Figure 1. Chromatogram of lavender essential oil. Numbers of constituents: 1- α-pinene, 2- camphene, 3-sabinene, 4.β-pinene, 5-β-myrcene, 6-2-carene, 7- m-cymene, 8-D-limonene, 9- eucalyptol, 10-β-cis-ocimene, 11- trans- β-ocimene, 12- γ-terpinen, 13- terpinolen, 14- linalool, 15-camphor, 16-endo-borneol, 17-terpinen-4-ol, 18-α-terpineol, 19- linalyl acetate, 20-lavandulol acetate, 21-α-terpinyl aceate, 22-caryophyllene, and 23-cis-β-farnesene.

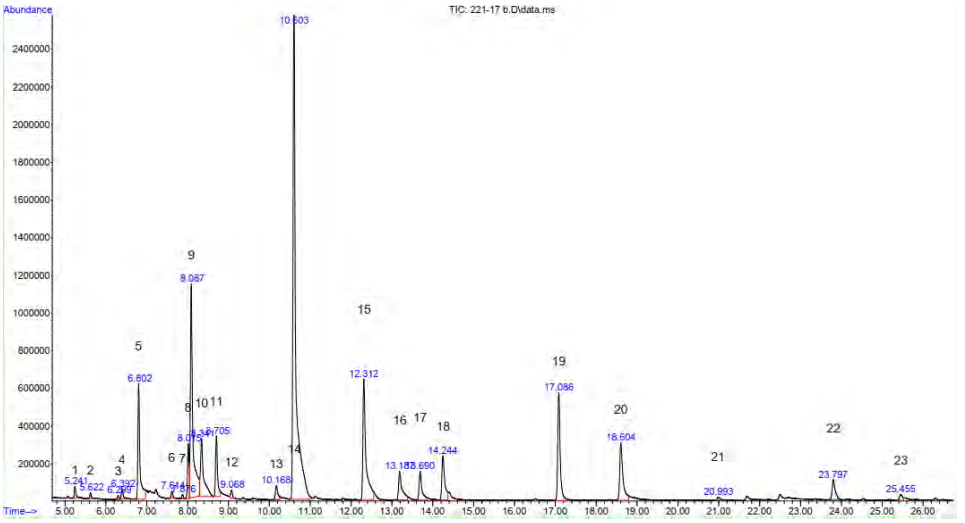

Figure 2. Chromatogram of lavandin essential oil. Numbers of constituents: 1-α-pinene, 2-camphene, 3-β-myrcene, 4-m-cymene, 5-D-limonene, 6-β-cis-ocimene, 7-trans- β-ocimene, 8-γ-terpinen, 9-terpinolen, 10-linalool, 11-1-octen-3-yl-acetate, 12-cosmene, 13-camphor, 14-endo-borneol, 15-lavandulol, 16-terpinen-4-ol, 17-α-terpineol, 18-n-hexyl butanoate, 19-linalyl acetate, 20-bornyl acetate, 21-lavandulol acetate, 22-caryophyllene, and 23-cis-β-farnesene.

Table 2. Chemical composition of essnetial oil lavender and lavandin cv grosso

№	Constituent name	Lavander essential oil, %	Lavandin cv. Grosso essential oil, %
1.	α- pinene	0.14	0.51
2.	camphene	0.18	0.21
3.	sabinene		0.16
4.	β-pinene		0.48
5.	β-myrcene	3.38	6.05
6.	2-carene		0.30
7.	m-cymene	0.17	0.16
8.	D-limonene	1.35	2.18
9.	eucalyptol		13.16
10.	β-cis-ocimene	6.50	4.56
11.	trans- β-ocimene	2.31	3.00
12.	γ-terpinen	0.25	0.37
13.	terpinolen	0.54	0.84
14.	linalool	64.47	35.40
15.	1-octen-3-yl-acetate	0.20	
16.	cosmene	0.38	
17.	camphor	0.35	9.54
18.	endo-borneol	1.18	2.48
19.	lavandulol	0.18	
20.	terpinen-4-ol	4.77	2.07
21.	α-terpineol	1.73	3.18
22.	n-hexyl butanoate	0.93	
23.	linalyl acetate	4.22	6.58
24.	bornyl acetate	0.17	
25.	lavandulol acetate	2.85	4.63
26.	α-terpinyl aceate		0.19
27.	caryophyllene	1.53	1.56
28.	cis-β-farnesene	0.51	0.46
	Σ (%)	**98.29**	**98.06**

However, in our research, the content of linalool in lavender essential oil was slightly higher than the mentioned authors' results. According to Alizadeh and Aghaee (2016) these differences may arise due to cultivars, environmental conditions, harvest time, essential oil extraction processes, and determination methodology. In addition to the linalool content, linalyl acetate is a crucial constituent determining the quality of lavender essential oil (Pokajewicz *et al*., 2021). In our research, its content ranged from 6.58% (*Lavanin cv*. grosso) to 4.22% (lavender). However, compared to previous studies (Pokajewicz *et al*., 2021), this study found a relatively small amount of linalyl acetate. This difference can be attributed to partial decomposition during hydrodistillation (Danh *et al*., 2013). Furthermore, the analyzed essential oils also differed in their camphor content. Lavender had a concentration of 0.35% camphor, while

lavender had a concentration of 9.54%. It should also be noted that a significant difference between the analyzed essential oils existed in the eucalyptol content. This constituent was found in significant concentrations in lavender essential oil (13.16%), while eucalyptol was not identified in lavender essential oil (0%). According to Kara and Baydar (2013) lavender essential oil is of higher quality in the perfume industry than Lavender essential oil because of its low camphor and eucalyptol level. The same authors believe that the camphor content in quality lavender oil must be between 0.5 and 1% in lavender and between 5.0 and 10.0% in lavender.

CONCLUSIONS

This is the first research on lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina. Research has shown that lavender has a high content of phenolic compounds, which is especially interesting if it is a source of antioxidants in the human diet. Furthermore, the research cultivars had a high yield of essential oil. Linalool dominated in the essential oil of both cultivars, especially lavender. The essential oil of lavender was rich in linalool and β-cis-ocimene but poor in camphor. On the hand, lavendin essential oil was rich in linalool, eucalyptol and camphor.

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RESPONSE OF *TRITICUM AESTIVUM* SEEDLINGS OF DIFFERENT ECOLOGICAL AND GEOGRAPHICAL ORIGIN TO HEAT AND DROUGHT: RELATIONSHIP WITH RESISTANCE TO OXIDATIVE STRESS AND OSMOLYTE ACCUMULATION

SUMMARY

The phenomenon of plant cross-tolerance to various stressors, particularly heat and drought, has been studied in considerable detail. However, there are no data on the relationship between resistance to these stressors in *Triticum aestivum* cultivars of different ecological and geographical origins at the stage of etiolated seedlings. At the same time, they are used for an accelerated assessment of the heat and drought resistance of breeding samples (separately for each factor). This work compared the response of seedlings of seven winter common wheat cultivars to heat stress (4-hour heating at 45°C) and a model drought (action of 12% PEG 6000). A correlation was found between the inhibition of seedling biomass accumulation as a whole (r=0.55) and separately for shoots (r=0.66) under heat and osmotic stress. A high correlation was shown between inhibition of shoot growth and accumulation of hydrogen peroxide and lipid peroxidation (LPO) products in shoots during heating (r=0.91 and 0.76, respectively) and a much lower correlation between the values of these markers of oxidative stress and inhibition of shoot growth during drought. A significant inverse correlation was found between the accumulation of sugars in the shoots and inhibition of shoot growth under drought (r=-0.85), and moderately high under heat stress (r=-0.60). At the same time, only a positive medium correlation (r=0.49) was observed between proline content and growth inhibition under both types of

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stress. However, a high positive correlation was found between proline and LPO products under drought conditions (r=0.91). It is concluded that the resistance of wheat seedlings to oxidative stress is more closely related to heat tolerance than to drought tolerance. The results also indicate a significant contribution of sugars, but not proline, to the resistance of wheat seedlings to drought and heat stress.

Keywords: *Triticum aestivum*, heat resistance, drought resistance, cross-tolerance, oxidative stress, sugars, proline

INTRODUCTION

The effects of drought and high temperatures are major factors limiting the productivity of most crops, especially cereals (Dudziak *et al*., 2019; Kolupaev and Blume, 2022). For example, each degree increase in temperature causes a reduction in wheat yields of around 6.0% (Itam *et al*., 2020). Drought causes an even greater drop in productivity: a 40% drop in available water from optimum results in a 20-40% drop in cereal yields (Daryanto *et al*., 2016).

In general, drought and high temperatures are considered the main abiotic stressors affecting wheat production (Aberkane *et al.,* 2021). There is evidence of greater sensitivity of *Triticum aestivum* than *T. durum* to these factors (Kavita *et al*., 2016). And it is known that resistance to the combination of drought and high temperatures is genetically different from tolerance to each of these factors (Keleş and Öncel, 2002). In turn, the properties of drought resistance and heat resistance are interrelated but far from identical (Cairns *et al*., 2013; Pržulj *et al.,* 2020).

To date, a number of statements have been formulated to explain the mechanisms of cross-resistance in plants to various stressors, including the effects of high temperatures and drought. These include the universal principles of activating the expression of protective genes by a limited set of signaling mediators (Bowler and Fluhr, 2000; Zhang *et al.,* 2020), control by the same transcription factors of a number of genes important for resistance (Pastori and Foyer, 2002), common reasons for cell damage caused by stressors of different nature (Iseki *et al*., 2014; Kolupaev *et al.*, 2020b), and polyfunctionality of some components of stress-protective systems (Szabados and Savoure, 2009; Kolupaev *et al*., 2020b). The latter refers to important stress metabolites such as proline and sugars, which can act as osmolytes and simultaneously exert antioxidant, membrane-protective, and anti-denaturation effects on cellular structures (Morelli *et al.,* 2003; Laxa *et al*., 2019).

An important cause of plant damage, when exposed to heat, is thought to be an excessive increase in reactive oxygen species (ROS) in cells (Asthir, 2015). The oxidative stress effect that develops under heat stress is a consequence of increased fluidity of chloroplast and mitochondrial membranes, leading to impaired electron transport in chloroplasts and mitochondria (Yoshioka, 2016; Choudhury *et al*., 2017). The effects of the activation of ROS-generating enzymes (primarily NADPH oxidase) under heat stress have also been shown (Gautam *et al*., 2017). Drought has a similar effect on plants. Restriction of CO_2 intake into cells due to the closing of stomata has an effect of over-reduction of

the electron-transport chain of chloroplasts and increases the probability of ROS formation (de Carvalho, 2008). Mitochondria are also known to contribute significantly to the development of oxidative stress under drought conditions. Thus, in wheat leaves under severe drought conditions, the content of carbonylated proteins in mitochondria was an order of magnitude higher than in chloroplasts (Bartoli *et al*., 2004). A correlation was shown between the stability of membranes of different rice genotypes under the action of osmotic stress agent polyethylene glycol (PEG) and the oxidative stress inducer methyl viologen (Iseki *et al*., 2014).

Despite many years of research on the tolerance of wheat to the combined adverse effects of high temperatures and drought (Schmidt *et al*., 2020; Tyagi and Pandey, 2022), data on the correlations between heat-stress and drought tolerance in varieties of different ecological and geographical origin at the seedling stage are still lacking. At the same time, results were obtained indicating a link between the resistance of etiolated seedlings to osmotic and heat stress and the field resistance of adult plants to these factors (Oboznyi *et al*., 2013; Pykalo *et al.,* 2020). With this phenomenon in mind, methods have been developed for evaluating the drought tolerance of wheat and other cereal varieties using the indicator of growth inhibition in the presence of osmotic agents (Pykalo *et al.,* 2020). There is also a method for assessing the heat resistance of wheat by inhibiting the growth of etiolated seedlings after exposure to elevated but not lethal temperatures (Pat. 45879 UA, 2002).

In this connection, studying the correlation between heat and dehydration resistance of etiolated wheat seedlings of different varieties can help to improve methods of evaluating samples of genetic collections and breeding material for cross-tolerance to these stress factors. A simultaneous study of the oxidative stress development in seedlings exposed to heat or model drought will make it possible to assess its role in damage development and to gain a deeper understanding of plant adaptation to these two most common natural stressors.

The purpose of this work was to compare the growth response of seedlings of seven common wheat cultivars of different ecological and geographical origin to heat stress and model drought created using PEG 6000. In addition, a comparative study of the development of oxidative stress and osmolyte accumulation under these adverse conditions was included in the scope of the work.

MATERIAL AND METHODS

Plant materials and treatments

Seven cultivars of common winter wheat (*Triticum aestivum* L.) of different ecological and geographical origin were used for the research. Five of these cultivars have been developed in different soil and climate zones in Ukraine. The Antonivka and Lira odeska (originated by Plant Breeding and Genetics Institute of the National Academy of Agrarian Sciences of Ukraine, Odesa) have the resistance required for cultivation in the Steppe zone (Khakhula

et al., 2013). The Doskonala cultivar (originated by Yuriev Plant Production Institute of NAAS of Ukraine, Kharkiv) is mainly intended for cultivation in the Forest-steppe and has a low heat and drought tolerance (Karpets *et al*., 2016). The Darynka kyivska and Bogdana (originated by Institute of Plant Physiology and Genetics of NAS of Ukraine, Kyiv) are characterized as moderately resistant and designed for cultivation in different zones (Chernobai *et al*., 2013, 2019; Khomenko, 2020). The cultivar Tobak (originator: Saaten-Union GmbH, Isernhagen HB, Germany), intended for cultivation in Central Europe but capable of maintaining productivity under conditions of drought and high temperatures, was also used in the research (Hlaváčová *et al*., 2018; Urban *et al*., 2018). In addition, the Avgustina cultivar, developed for cultivation in Belarusian Polesie, which is characterized by high humidity (originator: Scientific and Practical Center of NAS of Belarus), was used.

The experiments were carried out in the Laboratory of Plant Physiology and Biochemistry of the Yuriev Plant Production Institute of NAAS of Ukraine. Seed samples were provided by the National Center for Genetic Resources of Plants of Ukraine. Seeds of 2020 and 2021 reproduction years were used for the research.

The seeds were disinfected with a 1% sodium hypochlorite solution for 15 minutes and then washed with distilled water at least 8 times. For experiments on the effect of heat stress on seedling growth and biochemical parameters, seeds were germinated at 24°C for 3 days in Petri dishes on two layers of filter paper moistened with distilled water. Three-day-old seedlings of experimental variants were placed in open Petri dishes in a thermostat at 45±1°C (Pat. 45879 UA, 2002) and an air humidity of 40-45% (4 h exposure). To prevent the roots from drying out, a filter paper in the dishes was dampened with an equal amount of distilled water every hour. After exposure, one part of the seedlings was used for biochemical analyses, and the other part was placed in a 24°C thermostat to evaluate the growth response. The seedlings of control variants were kept in a thermostat at 24°C throughout the experiment. 24 h after heat stress, the inhibition of shoot and root growth of the seedlings was determined according to the formula:

$$I = \frac{(C_2 - C_1) - (E_2 - E_1)}{C_2 - C_1} \cdot 100\%$$

where I is growth inhibition (%); C1 and C2, E1 and E2 are, respectively, the initial and final values of seedling organ fresh weights in the control and experimental (heat stress) variants.

In experiments on the effect of model drought (osmotic stress) on seedling growth, seeds of experimental variants were germinated at 24°C in Petri dishes on two layers of filter paper moistened with 12% PEG 6000 solution. The seeds of the control variant were germinated under the same conditions in distilled water. On the 4th day of the experiment, biochemical analyses were carried out

and the shoot and root biomass of the seedlings was assessed. Inhibition of seedling growth under drought conditions was calculated using the formula:

$$I = 100 – (E/C \cdot 100\%),$$

where *I* is growth inhibition (%); C and E are the values of seedling organ fresh weight in the control and experimental (drought) variants, respectively.

The stress intensity causing a 20-60% inhibition of seedling growth in different cultivars was selected based on preliminary experiments using PEG 6000 solutions with concentrations in the range of 10-16%.

Determination of water content

The water content of seedling organs was determined gravimetrically by drying a sample of shoots or roots (0.5 g of fresh material) at 103°C to a constant weight.

Evaluation of hydrogen peroxide content

For the determination of H_2O_2 content, seedling shoots were homogenized in cold with 5% trichloroacetic acid (TCA). Samples were centrifuged at 8000 g for 10 min at 2-4°C on an MPW 350R centrifuge (MPW MedInstruments, Poland). Supernatant concentration of H_2O_2 was determined by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this, 0.5 ml of 2.5 M potassium thiocyanate, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ammonium iron(II) sulfate were added to tubes. After stirring, the samples were poured into cuvettes and the absorbance at 480 nm was determined.

Evaluation of LPO products content

The rate of lipid peroxidation (LPO) in seedling shoots was assessed by its products reacting with 2-thiobarbituric acid (TBA) (mainly malondialdehyde, MDA) (Kolupaev *et al*., 2020a). Sample material was homogenised in 0.1 M Tris-HCl buffer (pH 7.6); a 0.5% solution of TBA in 20% TCA was then added to the homogenate. Following heating the mixture in a boiling water bath for 30 min, the samples were cooled and centrifuged at 8000 g for 10 min. Afterwards, the absorbance of the supernatant was measured at 532 nm. Also the non-specific absorbance at 600 nm was determined, with the value subtracted from the main result. The measurements were carried out relative to a reagent mixture not containing TBA.

Assessing content of osmolites

The total sugars content in plant material was determined by the Morris-Roe method based on the anthrone reagent (Zhao *et al.,* 2003) in our modification. Sugars from the plant material were extracted with distilled water and heating for 10 minutes in a boiling water bath. The obtained extract was clarified by adding equal volumes (0.3 ml) of 30% zinc sulfate and 15% blood yellow salt to the tubes, then filtered through a paper filter and, if necessary, diluted several times with distilled water before measurement. 3 ml of anthrone reagent and 1 ml of the filtrate were added to the reaction tubes, and distilled water was added to the control sample instead of the filtrate. After boiling for 7

min in a water bath the samples were cooled and the absorbance was determined at 610 nm relative to the control solution. The standard used was D-glucose.

Proline content in the shoots was determined according to Bates *et al.* (1973) with modifications. Proline extraction from the plant material was done with distilled water and boiling for 10 min. Then the extract was filtered, and mixed in equal volumes with ninhydrin reagent and glacial acetic acid, and the samples were boiled in a water bath for 1 hour. The absorbance of the colored reaction product was determined at 520 nm using L-proline as a standard.

Replicate of experiment and statistical analysis

The experiment had 3 biological replicates. For the assessment of seedling organ weights and water content, each biological replication consisted of 30 seedlings. When measuring biochemical parameters, each sample of plant material was taken from 12-15 seedlings.

The results were statistically processed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. The figures and table show the mean values from three biological replicates and their standard errors. Different letters denote values with differences significant at $P \leq 0.05$. Correlation coefficients were estimated using the R programming language version 4.1.1 (R Core Team).

RESULTS AND DISCUSSION

Seedling growth under heat stress and drought

The most significant growth inhibition after 4 h of high temperature was observed in the seedlings of the cultivars Doskonala, Avgustina, and Bogdana (Table 1). It was lower in Darynka kyivska and Lira odeska. Tobak retained its ability to grow sufficiently well after stress and, finally, Antonivka showed the greatest resistance to heat.

Table 1. Effects of heat stress and drought on wheat seedling growth

Cultivar	Growth inhibition, %					
	Heat stress (45°C, 4 h)			Drought (12% PEG 6000)		
	Seed-lings	Shoots	Roots	Seed-lings	Shoots	Roots
Tobak	27.6±2.2d*	38.6±2.9c	11.6±0.4f	32.5±0.4d	37.9±0.5c	27.7±1.0c
Antonivka	16.4±1.3e	15.1±1.5d	17.8±2.1e	39.7±2.1bc	44.7±2.1b	35.6±2.2b
Lira odeska	40.7±1.2c	48.5±1.9b	29.0±2.2d	38.0±1.6c	44.0±2.2b	32.3±1.5b
Darynka kyivska	39.5±0.7c	42.7±1.8c	29.3±1.3d	41.1±1.3b	44.9±1.2b	37.1±1.6a
Bogdana	56.0±2.5b	61.1±2.5a	51.0±3.2b	39.2±0.7bc	50.6±1.3a	26.2±0.3c
Avgustina	51.6±2.7b	60.0±3.2a	43.7±2.5c	46.4±0.4a	52.9±1.0a	38.9±1.8a
Doskonala	61.2±1.0a	60.9±1.5a	62.6±2.1a	43.2±2.2ab	54.1±2.5a	32.0±2.8b

*Different letters in the same column denote statistically significant difference at $P \leq 0.05$

Shoot growth after heat stress was inhibited more severely than root growth in most cultivars (except Antonivka). This may in part be due to the loss of moisture by the shoots under heat stress, whereas no such effect was observed in the roots in any of the cultivars (Table 2). Variety differences in heat stress inhibition of shoot biomass growth were virtually indistinguishable from those of seedlings as a whole (Table 1).

Table 2. Water content (%) in the organs of wheat seedlings under heat stress and drought

Cultivar	Heat stress (45°C, 4 h)				Drought (12% PEG 6000)			
	Shoots		Roots		Shoots		Roots	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tobak	88.0 ± 0.2b*	87.8 ± 0.3bc	90.9 ± 0.3ab	90.8 ± 0.4ab	88.6 ± 0.4bc	87.8 ± 0.4c	91.2 ± 0.1ab	87.2 ± 0.2d
Antonivka	89.6 ± 0.3a	88.9 ± 0.4ab	90.2 ± 0.4b	90.2 ± 0.3b	90.0 ± 0.3ab	87.2 ± 0.3cd	90.5 ± 0.2b	85.9 ± 0.2f
Lira odeska	89.2 ± 0.2a	88.6 ± 0.3ab	90.2 ± 0.2b	90.0 ± 0.4b	89.5 ± 0.3b	87.8 ± 0.5c	90.6 ± 0.5b	86.8 ± 0.2c
Darynka kyivska	89.9 ± 0.3a	88.6 ± 0.3ab	90.4 ± 0.3b	90.1 ± 0.4b	90.7 ± 0.2a	88.1 ± 0.2c	90.7 ± 0.6b	87.8 ± 0.2d
Bogdana	88.8± 0.2 ab	87.4 ± 0.3bc	90.0 ± 0.2b	90.2 ± 0.2b	89.2 ± 0.6b	87.3 ± 0.7d	88.8 ± 0.1c	82.6 ± 0.2g
Avgustina	89.7 ± 0.3a	87.9 ± 0.2bc	91.3 ± 0.3a	91.1 ± 0.4a	90.1 ± 0.2ab	87.6 ± 0.1d	91.6 ± 0.2a	86.9 ± 0.1e
Doskonala	89.2 ± 0.2a	86.6 ± 0.3c	91.1 ± 0.4a	90.7 ± 0.3ab	89.8 ± 0.1ab	87.1 ± 0.5d	91.0 ± 0.3ab	85.6 ± 0.1f

*Different letters denote statistically significant difference at $P \leq 0.05$

Root growth was found to be the least sensitive to heat stress in Tobak and Antonivka cultivars. The inhibition of root growth was more pronounced in Lira odeska and Darynka kyivska. Strong suppression of root growth was observed in Doskonala, Bogdana, and Avgustina (Table 1).

The pattern of effects of model drought on seedling growth had both significant similarities and differences compared with the impact of heat stress. The most significant inhibition of seedling growth in general was observed in the cultivars Avgustina and Doskonala (Table 1). A lesser growth-inhibiting effect of drought was observed in Antonivka, Lira odeska, Darynka kyivska, and Bogdana. The seedlings of Tobak variety retained the greatest degree of growth capacity under PEG 6000 conditions (Table 1). Approximately the same varietal differences were observed for the shoot growth rate. The pattern of root growth inhibition during drought was slightly different. This effect was most noticeable in the cultivars Avgustina and Darynka kyivska. To a lesser extent root growth was affected by the drought in Doskonala, Lira odeska, and Antonivka. The least significant inhibition of root growth under model drought was observed in Bogdana and Tobak (Table 1).

Water content of seedling organs

As already noted, heat stress had almost no effect on the water content of the seedling roots (Table 2). Its effect on the shoot water content in the Tobak, Antonivka, Lira odeska, and Darynka kyivska cultivars was also insignificant. Some reduction in the water content was recorded in the shoots of Doskonala, Avgustina, and Bogdana.

Drought stress had a more pronounced effect on the water content of the seedling organs. Thus, a significant reduction in root water content was observed in all cultivars (Table 2). It was sharpest in Bogdana cultivar. The water content of the shoots also decreased significantly in all cultivars except the Tobak one.

Correlations between inhibition of seedling growth by heat and drought action.

Under heat stress, root and shoot growth inhibition were closely correlated with each other (r=0.81), indicating proportional inhibition of seedling organ growth in different cultivars (Figure 1). At the same time, the correlation between the inhibition of root and shoot growth under osmotic stress was low (r=0.24). This is due to the unequal effect of drought on root and above-ground seedling growth in different cultivars.

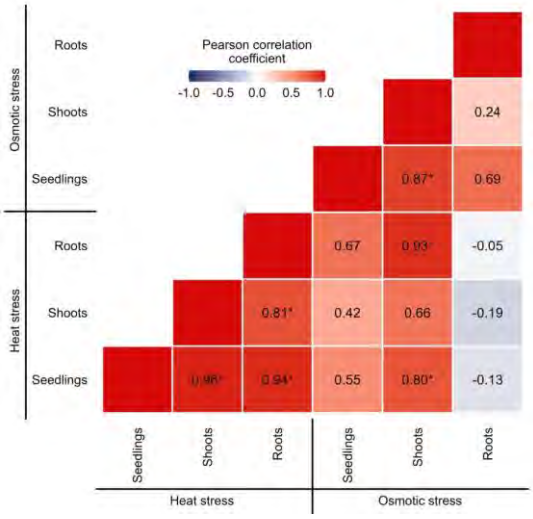

Figure 1. Correlation coefficients between growth inhibition of wheat seedlings and their organs under the effects of high temperature and drought.
*– significant at $P \leq 0.05$

A medium strength correlation (r=0.55) was observed between the total inhibition of seedling growth under heat and osmotic stress. Relatively high, although not significant at $P \leq 0.05$, was the correlation between shoot growth inhibition under the two types of stress (r=0.66). Herewith a significant correlation was found between the inhibition of root and seedling biomass

accumulation under heat stress and the inhibition of shoot biomass growth under drought (r=0.93 and 0.80, respectively). These values also indicate, to some extent, a cross relation between heat and drought tolerance of seedlings. At the same time, no correlation was found at all between the inhibitions of root growth under these stresses (Figure 1).

The ability to accumulate sufficient above-ground biomass under stress conditions is thought to be a more important characteristic of resistance than root growth (Laxa *et al.,* 2019). In terms of inhibition of shoot growth after heat stress, the cultivars studied were ranked as follows: Antonivka < Tobak < Darynka kyivska < Lira odeska < Avgustina ≤ Doskonala ≤ Bogdana. At the same time, the order of the cultivars differed somewhat in terms of the magnitude of shoot growth suppression under drought conditions: Tobak < Lira odeska ≤ Antonivka ≤ Darynka kyivska < Bogdana ≤ Avgustina ≤ Doskonala. Despite such differences, it can be observed that the cultivars Tobak, Antonivka, Darynka kyivska, and Lira odeska showed significantly greater resistance to both stresses compared to Avgustina, Bogdana, and Doskonala.

Indicators of oxidative stress in shoots under the action of heat and drought

After 4-h heating of the seedlings, a significant increase (1.5-2.1 times) in hydrogen peroxide content was recorded in the cultivars Doskonala, Bogdana, and Avgustina (Table 3). In Lira odeska and Darynka kyivska, this effect was much weaker, while in Tobak and Antonivka, the hydrogen peroxide content after seedling heating was almost unchanged. The content of the LPO product MDA as well as the amount of H_2O_2 increased most markedly (1.3 times) in the cultivars Doskonala, Bogdana, and Avgustina (Table 3). A smaller increase in this index after exposure to high temperature was recorded in Lira odeska and a very slight increase in the cultivars Tobak and Antonivka.

Table 3. Effect of heat and osmotic stress on hydrogen peroxide and MDA content in wheat seedling shoots

Cultivar	Content, % to control			
	Heat stress (45°C, 4 h)		Drought (12% PEG 6000)	
	H_2O_2	MDA	H_2O_2	MDA
Tobak	106±4d	107±2c	103±18b	126±7c
Antonivka	102±3d	103±3c	89±5c	124±2c
Lira odeska	119±5c	115±4b	91±17c	129±4c
Darynka kyivska	112±4cd	108±3bc	158±15ab	123±1c
Bogdana	157±4b	130±4a	109±14bc	131±7c
Avgustina	150±6b	130±2a	131±15b	180±4a
Doskonala	209±2a	130±2a	176±9a	143±1b

*Different letters in the same column denote statistically significant difference at $P \leq 0.05$

Under drought conditions, the H_2O_2 content increased markedly in the cultivars Doskonala, Darynka kyivska, and Avgustina and less so in the Bogdana. In the Tobak, Antonivka, and Lira odeska cultivars, however, the hydrogen peroxide

content did not change significantly. The MDA content increased most significantly in Avgustina and Doskonala under the influence of drought (Table 3).

Osmolyte content in shoots under heat and drought conditions

The initial proline content in 3-day-old seedlings (heat stress experiment) and 4-day-old seedlings (drought experiment) differed slightly, with a tendency to increase in 4-day-old seedlings (Table 4). No significant relationship was observed between its content in the control and resistance to the relevant stressors. For example, the highest proline content was found in the heat and drought-non-resistant cultivar Avgustina. On the other hand, the proline content was almost the same in the Darynka kyivska, which was sufficiently resistant to these stresses, and the least resistant Doskonala. Heat stress caused a 2-4-fold increase in proline content in all cultivars. The highest amounts were recorded in the non-resistant cultivars Bogdana and Avgustina (Table 4). The increase in proline content during drought was less pronounced than during heat stress, with the highest content also found in the Avgustina cultivar.

Table 4. Proline and sugars content in shoots of etiolated wheat seedlings under heat stress and drought

Cultivar	Heat stress (45°C, 4 h)				Drought (12% PEG 6000)			
	Proline (mg/g dry weight)		Sugars (mg/g dry weight)		Proline (mg/g dry weight)		Sugars (mg/g dry weight)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tobak	0.94 ± 0.02g*	1.86 ± 0.05e	210 ± 7f	273 ± 6c	1.12 ± 0.08e	1.59 ± 0.06cd	197 ± 7de	300 ± 22a
Antonivka	0.71 ± 0.01h	2.71 ± 0.09c	215 ± 7f	314 ± 5b	0.85 ± 0.05f	1.04 ± 0.05ef	200 ± 9d	259 ± 10b
Lira odeska	0.79 ± 0.03gh	2.43 ± 0.08d	238 ± 10de	309 ± 6b	0.90 ± 0.08f	1.24 ± 0.11de	199 ± 8de	259 ± 4b
Darynka kyivska	1.23 ± 0.04f	2.46 ± 0.08d	208 ± 7f	363 ± 6a	1.44 ± 0.05d	1.84 ± 0.10bc	211 ± 3d	223 ± 3cd
Bogdana	1.33 ± 0.06f	3.40 ± 0.08a	207 ± 3f	251 ± 7d	1.37 ± 0.10de	1.70 ± 0.15c	182 ± 10e	210 ± 10d
Avgustina	1.89 ± 0.04e	3.23 ± 0.08b	244 ± 7d	252 ± 9d	1.93 ± 0.05b	3.70 ± 0.24a	204 ± 6d	232 ± 9c
Doskonala	1.15 ± 0.01f	2.79 ± 0.12c	206 ± 5f	229 ± 9e	1.49 ± 0.21d	1.61 ± 0.10c	213 ± 9cd	215 ± 14cd

*Different letters denote statistically significant difference at $P \leq 0.05$

The sugar content of 3-4 day-old seedlings differed slightly depending on age and cultivar (Table 4). Heat stress caused an increase in sugar content of the shoots of all the cultivars studied with the exception of Avgustina. Wherein higher absolute values were recorded for the more heat-resistant cultivars (Darynka kyivska, Antonivka, Lira odeska, and Tobak). Under the drought conditions created by PEG 6000, a significant increase in sugar content was found in the resistant cultivars Tobak, Antonivka, and Lira odeska (Table 4). In the relatively resistant Darynka kyivska, as well as in the weakly resistant

Bogdana and Avgustina, this effect was relatively small, while in the cultivar Doskonala it was absent.

Correlations between growth inhibition and the content of oxidative stress markers and osmolytes in shoots

A strong correlation (r=0.76 and 0.91, respectively) was found between inhibition of shoot growth under heat stress and its hydrogen peroxide and MDA content (Figure 2). At the same time, the corresponding correlation coefficients were much lower for drought conditions (Figure 2). There is therefore reason to believe that under heat conditions the contribution of oxidative stress to damage and inhibition of seedling growth is more significant than under drought conditions.

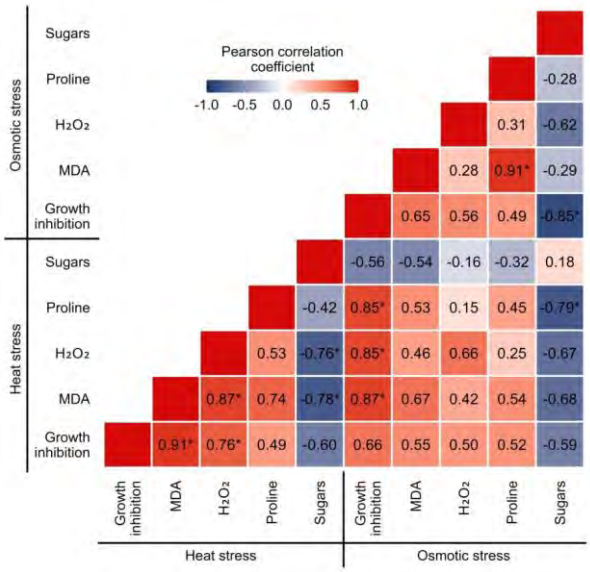

Figure 2. Correlation coefficients between shoot growth inhibition and content of oxidative stress markers and osmolytes under high temperature and drought stress. *–significant at P≤0.05

A moderate positive correlation (r=0.49) was found between the inhibition of shoot growth under heat stress and proline content, but not significant at P≤0.05 (Figure 2). At the same time, a fairly strong negative correlation (r=-0.60) was shown between shoot growth inhibition and sugar content. Also of note is the negative correlation, significant at P≤0.05, between sugar content and oxidative stress (MDA and H_2O_2 content) after heating the seedlings. This fact may indicate a role for sugars in protecting seedlings against oxidative stress under high-temperature conditions. It was reported that soluble carbohydrates can participate in antioxidant protection under heat stress and are probably involved in antioxidant system regulation (Kolupaev *et al*., 2023). For example, exogenous glucose increased heat tolerance and reduced ROS in cucumber plants (Huang *et*

al., 2015). This was accompanied by an increase in the number of transcripts and increased activity of Cu/Zn-SOD, Mn-SOD, catalase, and glutathione reductase. The heat-tolerant chickpea cultivar accumulated more sugars under stress compared to the non-tolerant one (Yadav *et al.,* 2022). It is also known that sucrose can protect membranes by forming bonds with phosphate "heads" of lipids (Tarkowski and Vanden, 2015).

In our experiments under drought conditions, as well as after heat stress, a positive correlation of moderate strength was found between proline content and growth inhibition (Figure 2). Note the very high level of positive correlation between proline content and the main marker of oxidative stress MDA in shoots during drought (r=0.91). In this connection, it can be assumed that under these experimental conditions, the proline content correlates not with the resistance of the seedlings to the stress factor but, on the contrary, with the manifestation of oxidative damage. Similar effects have been found in studies of the impact of other stressors on plants. For example, a strong positive correlation has been shown between proline and MDA accumulation when rapeseed plants were exposed to a wide range of toxic copper concentrations (Kholodova *et al*., 2018). It is difficult to give an unambiguous interpretation of such effects. On the one hand, they may indicate a link between oxidative stress and proline accumulation (Signorelli *et al.*, 2014). On the other hand, this relationship cannot be unequivocally interpreted as proline's involvement in antioxidant defense. High proline concentrations are known in some cases to induce pro-oxidative processes in mitochondria and may induce programmed cell death (Fabro *et al*., 2004; Miller *et al*., 2009). To discuss this issue is beyond the scope of the results obtained in this work. It should be noted, however, that the presence of both antioxidant and pro-oxidant effects of proline casts doubt on its use as an indicator of stress tolerance, at least in large-scale experiments to evaluate breeding material (Pykalo *et al*., 2020).

While proline accumulation in wheat seedlings under drought conditions correlated positively with growth inhibition, the content of other osmolytes, sugars, was in a high negative correlation with growth inhibition (r=-0.85). This fact indicates the special role of sugars as osmolytes and probably as multifunctional protective compounds in seedling adaptation to drought conditions.

In general, the results obtained indicate both common and specific features of wheat seedling adaptation to the two stressors that often act simultaneously in natural conditions – high temperature and drought. Seven cultivars of different ecological and geographical origins were used in our experiments. In their example, it was possible to establish a positive correlation between heat and drought resistance of the seedlings. However, the values of the correlation coefficients for this sample were not significant at $P \leq 0.05$. Perhaps if more correlation pairs were used, such a relationship would show up as more reliable. On the other hand, the presence of a significant correlation between oxidative

stress and growth inhibition only under heat stress but not under drought stress (Figure 2) indicates differences in the mechanisms of plant damage under these two stresses. At least within the scope of the model we used, it can be argued that heat damage is more dependent on ROS formation than PEG-induced drought damage. It should be noted that on etiolated seedlings of different cereal species, it was possible to show the relationship between heat tolerance and their resistance to oxidative stress (Kolupaev *et al*., 2022). The functioning of the enzymatic component of the antioxidant system has not been studied in the present work. However, a fair amount of data on the relationship between heat tolerance in plants and the functioning of their antioxidant defense components is generally available in the literature (Puckette *et al*., 2007; Kolupaev *et al*., 2023).

As for drought stress, osmolytes, in particular sugars, seem to play an especially important role in resistance under the conditions of the model we used. Sugars have been shown to be a better substitute for water under drought conditions and better preserve the hydrated state of proteins in drought compared to proline (Tyagi and Pandey, 2022).

Overall, the results show that etiolated wheat seedlings can be used both for accelerated evaluation of breeding material for heat and drought resistance and for studying the mechanisms of damage caused by these factors and adaptation to them. To elucidate the role of the antioxidant system in wheat resistance to the above stress factors, it is of further interest to study the response of varieties of different ecological and geographical origins to the action of direct oxidative stress agents – exogenous ROS or inducers of their formation. Similar studies carried out on clover (Puckette *et al*., 2007) and Arabidopsis (Iseki *et al*., 2014) plants from different ecological and geographical zones indicate a link between adaptation to unfavorable natural conditions and resistance to pro-oxidants.

CONCLUSIONS

A relationship has been established between the resistance of seven varieties of etiolated wheat seedlings of different ecological and geographical origin to high temperatures and drought. Wherein specific features of the action of each stressor were also found. A close correlation between the inhibition of seedling growth under heat stress and the accumulation of oxidative stress markers, hydrogen peroxide and MDA, in seedlings was shown. In a drought, such a connection was not so prominent. At the same time, the accumulation of osmolytes with multifunctional protective effects appears to be more important for seedling drought resistance. A high negative correlation was shown between sugars accumulation and inhibition of seedling growth in drought conditions. At the same time, proline accumulation was positively correlated with growth inhibition and the development of oxidative stress, which limits the use of this indicator in applied research to assess the stress tolerance of wheat varieties.

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CLIMATE CHANGE IMPACTS ON WATER BALANCE COMPONENTS IN BOSNIA AND HERZEGOVINA AND CROATIA

SUMMARY

The climate of Southeastern Europe, where Bosnia and Herzegovina (BiH) and Croatia are located, is changing in line with the global trends. The spatial and seasonal distribution of precipitation is changing, while the temperatures increased 0.4-0.8 ºC on average compared to 1961-1990, most notably during summer (1.0-1.2 ºC). Depending on the different Representative Concentration Pathway (RCP) scenarios, the temperatures in this area are projected to further increase by 1.7-4.0 °C. In order to understand the effects of climate change on regional water resources, it is important to assess the impacts of these changes on the components of the water balance. The aim of this study was to determine and compare the severity of changes in annual water balance between two climate periods (1961-1990 and 1991-2020). The results indicate that climate change has a different temporal and spatial effect. All areas showed a positive trend in mean air temperature (0.29-0.36 ºC per decade), reference evapotranspiration (5.96-32.14 mm per decade) while precipitation, total runoff, amount of snow and actual evapotranspiration vary depending on the location and time period. The key characteristic of the 1991-2020 period compared to 1961-1990 is the greater variation of all components of the water balance.

Keywords: climate change, soil water balance, soil moisture deficit, evapotranspiration

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INTRODUCTION

Analysis of meteorological parameters plays the important role in a changing environment, especially in agricultural water management. The trends of different meteorological parameters, such as temperature, precipitation and relative humidity have been the parts of many studies across the world (Gocic and Trajkovic 2013, Nikzad Tehrani *et al*. 2019, Pandey and Khare 2018).

In Bosnia and Herzegovina (BiH), a significant body of research is focused on the analysis of changes and increase in air temperature (Popov 2020, Popov *et al.* 2019, Popov *et al.* 2018, Popov, Gnjato *et al*. 2017), amount and distribution of precipitation (Popov *et al*. 2019, Popov *et al*. 2017, TNC 2016), as well as changes in the water balance (Čadro *et al*. 2020, Ljuša *et al*. 2020). As for Republic of Croatia, recent studies have been conducted to address the climate change phenomenon (Beslik and Causevic 2019) and to examine the main climate trends (Bilandžija, 2019, Bilandžija and Martinčić 2019, Cindrić *et al*. 2016, Čepo, 2021, Ferina *et al*. 2021, Marinović *et al.* 2021).

In many studies of climate change, the region where Bosnia and Herzegovina and Croatia are located is defined as the Western Balkans or Southeastern Europe. This area is under the great impact of global warming and highly vulnerable to climate change, with an observed temperature increase of 1.2ºC and projected to warm further by 1.7-4.0°C depending on global efforts in greenhouse gas (GHG) emission reduction or RCP models. This temperature increase would lead to a reduction in precipitation by up to 30% (Ali, *et al*., 2022).

BiH and Croatia are countries whose larger parts are located in continental and Mediterranean climate zones (Peel *et al*. 2007). These two zones differ in all basic characteristics, most notably in temperature and amount of precipitation (Bajić and Trbić 2016). The Mediterranean region is considered to be warming 20% faster than the global average (Ali, *et al*., 2022). Therefore, climate change has a different impact on these two climates.

Such negative trends will have an impact on agriculture as this is crucial for development and employment in BiH and Croatia. Agriculture is highly sensitive to climate conditions and it is expected to be strongly affected by climate change, potentially resulting in the loss of livelihoods and jobs Rüttinger, *et al.,* 2021). The aim of this study was to determine and compare the severity of changes in annual water balance between two climate periods (1961-1990 and 1991-2020) and two climate zones (BiH and Croatia), in order to analyze the impact of climate change on soil water balance.

MATERIAL AND METHODS

Research was carried out for the wider area of two countries - Croatia and BiH. Croatia is located at the crossroads of southeast and central Europe, Balkan peninsula, and southern Europe (45°8′30″N, 16°13′45″E). BiH is located in south-eastern Europe, at latitude 43°52' N and longitude 18°25' E. The basic country profiles are given in Table 1.

Table 1. Main characteristics of the study areas

Characteristic	Croatia	Bosnia and Herzegovina
Area (km^2)	56,594	51,129
Water (%)	1.09	1.40
Coastline (km)	1,777	20
Mean elevation (m)	331	500
Highest point (m)	1,831	2,386
GDP (nominal) per capita[1] ($)	17,337	7,078
Population	3,888,529[2]	3,531,159[3]
Population density (per km^2)	68.70	69.06
HDI[4] for 2022	0.837	0.769
Köppen climate classification[5]	Dfc, Dfb, Dfa, Cfb, Cfa, Csb, Csa	ET, Dfb, Cfa, Cfb, Csa
CRI[6] average for 2000–2019	47,00 (53)	68,17 (122)

[1]*Gross domestic product (IMF, 2019)*
[2]*Census of population, households and dwellings in the Republic of Croatia in 2021, source: Central Bureau of Statistics of the Republic of Croatia, 2021*
[3]*Agency for Statistics of Bosnia and Herzegovina, Census 2013 (Jukić, 2016).*
[4]*Human development index, source: United Nations Development Programme.*
[5]*Dfb - humid continental climate, Cfa, Cfb - temperate warm and humid climates, Csa - mediterranean climate and ET - tundra climate (Peel et al. 2007).*
[6]*Global Climate Risk Index (Eckstein et al. 2021)*

Both countries have similar Köppen climatic types (Geiger, 1961, Peel *et al*. 2007), area and population. The biggest difference is the large coastal area that Croatia has (1,777 m), ie the higher mean altitude (500 m) in BiH. BiH is mainly hilly to mountainous, with 5% is lowlands, 24% hills, 42% mountains, and 29% of karst area (TNC 2016, Žurovec *et al*. 2017).

The most recent Global Climate Risk Index (CRI) places BiH at 122 place, and Croatia at 53td in terms of exposure and vulnerability to extreme weather events (Eckstein *et al*. 2021).

Climate data

Ten weather stations (WS) with long-term continuous climate data records, were selected for this research, five in Croatia: Osijek (Čepin), Dubrovnik, Rijeka, Split (Marjan), and Zagreb (Maksimir); and five in BiH: Livno, Sanski Most, Sarajevo (Bjelave), Tuzla and Mostar. Daily weather data, including mean (T_{mean}), maximum (T_{max}) and minimum (T_{min}) air temperature, sum of precipitation (P), mean relative humidity (RH_{mean}), wind speed at 2 m height (u_2) and sunshine hours (n) for the period 1961-2020 were collected and averaged over each month.

The 60-year period is divided into two climatic periods, the climatic period of the reference normal: 1961-1990 and the last climatological standard normal 1991-2020 (WMO, 2017).

Data were provided by the Federal Hydrometeorological Institute Sarajevo and Croatian Meteorological and Hydrological Service. Basic location characteristics are shown in Table 2.

Table 2. Location and climate characteristics of 10 studied weather stations

WS	Country	A (m)	ºE	ºN	T_{mean} (ºC)	T_{max} (ºC)	T_{min} (ºC)	RH_{mean} (%)	u_2 (m s^{-1})	n (h)	P (mm)
Osijek	CRO	89	18.561	45.502	11.29	16.78	6.26	77.91	1.74	5.33	676
Dubrovnik	CRO	52	18.085	42.644	16.71	24.62	8.57	61,83	2.38	7.21	1157
Rijeka	CRO	120	14.442	45.336	14.17	23.81	4.88	62.80	1.66	6.09	1576
Split	CRO	122	16.426	43.508	16.40	24.35	7.61	50.03	2.79	7.23	814
Zagreb	CRO	123	16.033	45.821	11.07	23.98	-0.54	74.75	1.43	5.28	863
Livno	BiH	724	17.016	43.816	9.51	15.77	3.61	71.15	1.68	6.36	1153
Sanski M.	BiH	158	16.666	44.750	10.64	17.05	5.19	78.87	1.81	5.12	1042
Sarajevo	BiH	630	18.433	43.866	10.05	15.67	5.33	70.63	1.65	5.00	939
Tuzla	BiH	305	18.700	44.550	10.51	16.84	5.46	76.06	1.21	5.01	909
Mostar	BiH	99	17.800	43.350	15.09	20.47	10.53	62.02	2.48	6.44	1482

Note: A – altitude; ºE – longitude; ºN – latitude; T_{max} – mean maximum air temperature; T_{min} – mean minimum air temperature; RH_{mean} – mean relative humidity; u_2 – mean wind speed at 2m height; n - actual duration of sunshine; P - precipitation.

WS Dubrovnik, Rijeka and Split and Mostar are located in the area of the Mediterranean climate. WS Osijek, Zagreb, Livno, Sanski Most, Sarajevo and Tuzla are located in continental climate. Most of the selected stations are located in the urban zones of this area, and due to the development of these cities in the period from 1960 to 2020, the urban heat island (UHI) effect can be present. Given that there are no studies that determine the extent of this impact, nor are there any rural stations that meet the criteria, we can say that the selected WS best represent the selected climates in Croatia and Bosnia and Herzegovina.

Water balance calculation

Monthly water balance was calculated using the modified Thornthwaite-Mather method (Dingman, 2002, Thornthwaite and Mather 1955, Thornthwaite and Mather 1957) as shown in Figure 1. Thornthwaite-Mather method (TM) required data on monthly precipitation (P), average monthly air temperature (T), reference evapotranspiration (ET0) and soil available water content (SOILmax). The value SOILmax = 100 mm was used (McBean *et al*. 1995) since this is the most commonly used value for the types of soil that are found in the study locations (Čadro, 2019, Šimunić, 2013).

Dingman's (2002) modification of the Thornthwaite-Mather water balance includes calculation of the amount of snow (SNOW), which is determined based on a defined temperature threshold, i.e. the temperature at which precipitation reaches the ground in the form of snow (sn_threshold), P is divided into snow (SNOW) and rain (P_remain). This snow remains on the ground and is carried over to the next month (PACK). In the case of a temperature rise in one of the following months, the snow melts (MELT) and if possible, infiltrates the ground. Rain (P_remain) and snowmelt water (MELT) represent the total water input to the soil (W).

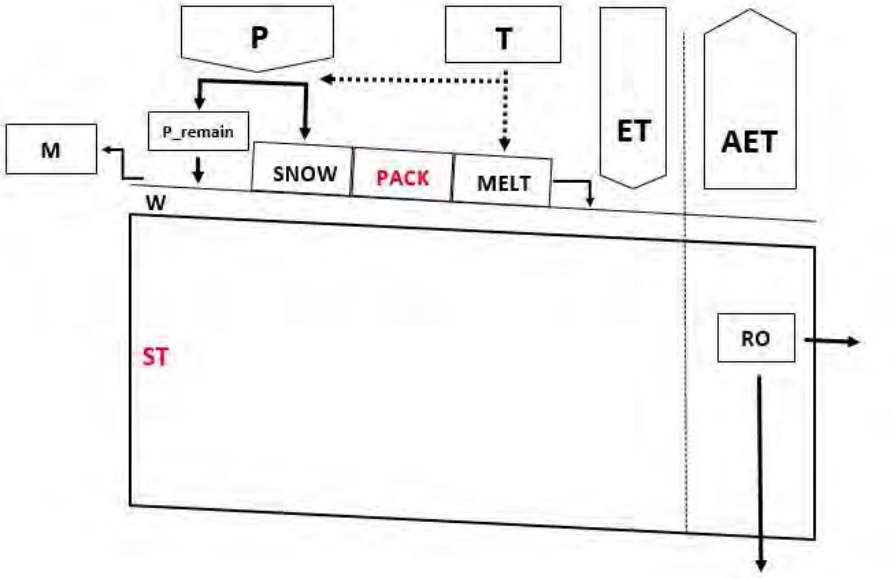

Figure 1. Graphic representation of Modified Thornthwaite-Mather method

Reference evapotranspiration (ET_0) was calculated using the standard fao-pm equation (Allen *et al*. 1998):

$$ET_0 = \frac{0.408\Delta \cdot (R_n - G) + \gamma \cdot \frac{900}{T_{mean} + 273} \cdot u_2 \cdot (e_s - e_a)}{\Delta + \gamma \cdot (1 + 0.34 \cdot u_2)} \quad (1)$$

where ET_0 is the reference evapotranspiration (mm day^{-1}), R_n is net radiation at the crop surface (MJ m^{-2} day^{-1}), G is the soil heat flux density (MJ m^{-2} day^{-1}), t_{mean} is mean daily air temperature at 2 m height (°c), u_2 is the wind speed at 2 m height (m s^{-1}), e_s is the saturation vapor pressure, e_a is the actual vapor pressure, e_s–e_a is saturation vapor pressure deficit, δ is the slope of the vapor pressure curve (kPa $°c^{-1}$) and y is the psychrometric constant (kPa $°c^{-1}$).

All necessary parameters required for the calculation of ET_0 were computed following the procedure developed in FAO-56 (Allen *et al.,* 1998) via REF-ET: reference Evapotranspiration Calculator (Allen & Zhenguli, 2016) software.

After the calculation of annual means (μ) and the standard deviation (σ) for all analysed water balance components, a statistical measure of the dispersion of data points and the coefficient of variation (CV) were calculated. To detect the trends (b) within annual time series of water balance components (Reference evapotranspiration – ET_0, actual evapotranspiration - AET, soil moisture deficit - SMD, total runoff - RO and SNOW) parametric method of linear regression was used.

The severity of climate change effects was analyzed for the period of 1961-1990, defined as "reference normal" (RN), and 1991-2020 defined as "current normal" (CN). All analyzed parameters are divided into climate parameters (CP) including T, P, RH_{mean}, n, and water balance components (WB) including calculated values of ET_0, AET, SMD, RO and SNOW.

RESULTS AND DISCUSSION

Climate parameters

The difference shown in the following tables is the result of subtracting all the values of the obtained parameters for the current climate normal (1991–2020) from the data values for the reference climate period (1961–1991). Positive changes are shown in red, and negative in blue. The intensity of the colour indicates the strength of the change. Air temperatures for the analysed period are shown in Table 3.

In the both climate periods, the highest mean monthly temperature (T_{mean}) is in Dubrovnik (RN 16.26 °C and CN 17.14 °C), and the lowest is in Livno (RN 8.93 °C and CN 10.08 °C). The coefficient of variation (CV) shows the largest temperature variations in the area of Tuzla and Sarajevo. An increase in temperature was found in all WS, ranging from 0.86 °C in Dubrovnik to 1.61 °C in Zagreb. In addition, an increase in variations of monthly air temperatures was found at all locations.

Table 3. Mean air temperatures (T_{mean}) and differences between the analysed periods

Air Temperature (°C)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	8.93	10.12	9.55	10.01	10.82	10.26	14.57	13.63	15.94	16.28
	σ	0.41	0.52	0.42	0.47	0.71	0.54	0.44	0.41	0.39	0.34
	CV	4.62	5.15	4.43	4.69	6.59	5.22	3.05	3.04	2.43	2.08
Current normal 1991-2020	$\bar{x}$	**10.08**	11.16	10.56	11.01	11.77	11.87	15.61	14.71	16.86	**17.14**
	σ	0.61	0.69	0.73	0.81	0.76	0.79	0.59	0.68	0.63	0.67
	CV	6.02	6.22	**6.96**	**7.35**	6.47	6.69	3.77	4.65	3.75	3.91
Difference	$\bar{x}$	1.15	1.04	1.01	1.00	0.95	**1.61**	1.05	1.09	0.92	0.86
	σ	0.19	0.17	0.31	0.34	0.05	0.26	0.14	0.27	0.24	0.33
	CV	1.40	1.07	2.53	2.65	-0.12	1.48	0.72	1.61	1.32	1.84

Such increases in air temperature were also recorded in other studies in BiH and Croatia. Annual temperature trends are positive and statistically significant throughout the territory of BiH. The values of this change, depending on the period of analysis and the location, vary between 0.16 and 1.2 °C per decade (Čadro *et al*. 2022, Čadro *et al.* 2020, Jurkovic *et al*. 2021, Popov, 2020, Popov *et al.* 2019, TNC 2016, Trbic *et al.* 2017).

Large temperature variations, especially in the continental climate, indicate an increase in the annual frequency of summer days and tropical days. Popov *et al.* (2018) found an increase in the annual frequency of summer days in the range of 3.0–8.2 days per decade and tropical days 1.2–8.1 days per decade.

Čepo (2021) claims that Croatia, as a Mediterranean country marks the average temperature increase of +1.54 °C and estimates that by 2040 it will be +2.2 °C compared to the pre-industrial level. According to Bilandžija and Martinčić (2019), in comparison to 1961-1990 period, the 1991-2018 period marks higher mean air temperatures followed by increased actual evapotranspiration and prolonged vegetation periods. Table 4 shows data for precipitation in the analysed periods.

Table 4. Mean annual precipitation (P) and differences between analysed periods

Precipitation (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	1144	1024	929	896	650	852	1516	1561	825	1199
	σ	161	125	143	123	102	136	297	229	138	257
	CV	14.08	12.22	15.40	13.79	15.77	15.98	19.58	14.66	16.72	21.45
Current normal 1991-2020	$\bar{x}$	1163	1061	948	923	**701**	874	1447	**1590**	801	1115
	σ	224	180	148	193	157	156	362	302	183	276
	CV	19.30	16.98	15.64	20.88	22.40	17.82	**25.01**	18.99	**22.83**	**24.70**
Difference	$\bar{x}$	18.73	37.76	19.00	27.37	51.34	21.70	**-68.26**	28.92	-24.57	**-83.14**
	σ	63.40	55.16	5.35	69.73	54.61	19.54	64.99	73.16	44.85	18.43
	CV	5.23	4.75	0.24	7.10	6.63	1.84	5.43	4.33	6.11	3.25

The highest annual amount of precipitation (P) is in Rijeka with 1590 mm, followed by Mostar with 1447 mm. The lowest amount of precipitation is in Osijek (701 mm). The largest variations in precipitation are in the Mediterranean part, i.e. in Mostar, Dubrovnik and Split. The area where the annual amount of precipitation decreased highest is in Dubrovnik (83 mm). All WS in the continental climate had an increase in annual precipitation, with the highest increase in Osijek, where the average annual precipitation in CN has increased by 51 mm. It is interesting to note that the coefficient of variation increased at all WS.

In contrast to the consistent warming trend found for the entire research area, changes in the precipitation regime did not show spatially and temporally coherent trends. Many other authors also came to similar results (Čadro *et al.* 2020, Popov, 2020, Popov *et al*. 2019, TNC 2016). Analysis for Croatia shows that at the state level, the average annual amount of precipitation has increased very little. However, the changes are very different depending on the region meaning that in most parts of Croatia, a significant decrease in the total amount of precipitation is observed, mainly during the summer, at the level of 7-2% per decade (Čepo, 2021).

Also, the result of our study shows that there are certain differences between the studied Mediterranean and continental areas. In the Mediterranean,

especially in the south (Mostar, Split and Dubrovnik), i.e. in locations with a large amount of annual precipitation (1115-1590 mm), there was a decrease (24-83 mm) while in the continental part the amount of precipitation increased (18-51 mm).

For the area of southern Croatia and BiH, this change, combined with increase in variability and extreme rainfall events (Popov *et al.* 2017), can cause significant problems, because even though there are large amounts of precipitation, most of them fall in the period when the vegetation is dormant. The summer period is very dry and this kind of change only deepens the problem of water supply for all sectors (agriculture, industry and domestic) during the summer months. Table 5 shows data for the relative air humidity in the analysed periods.

Table 5. Relative air humidity (RH_{mean}) and differences between analysed periods

Relative air humidity (%)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	68.99	79.48	71.19	77.58	79.35	76.57	62.04	62.88	58.41	63.24
	σ	6.24	1.33	2.76	2.65	4.18	1.64	3.13	2.33	2.16	2.14
	CV	9.04	1.67	3.88	3.42	5.27	2.14	5.04	3.70	3.70	3.38
Current normal 1991-2020	$\bar{x}$	73.31	**78.28**	70.08	74.53	76.47	72.93	62.00	62.73	**57.64**	60.41
	σ	8.02	2.04	3.05	2.69	2.76	2.06	3.96	3.12	2.45	3.52
	CV	**10.94**	2.60	4.35	3.61	3.61	2.82	6.39	4.97	4.26	5.82
Difference	$\bar{x}$	4.32	-1.20	-1.11	-3.05	-2.88	-3.64	-0.05	-0.16	-0.76	-2.83
	σ	1.78	0.71	0.29	0.04	-1.42	0.42	0.83	0.79	0.30	1.38
	CV	1.90	0.93	0.47	0.19	-1.66	0.69	1.35	1.27	0.56	2.45

The lowest values of mean relative humidity (RH_{mean}) are in the Mediterranean (Split, Dubrovnik, Mostar, Rijeka), and the highest in the continental part (Sanski Most, Osijek, Tuzla). WS Livno stands out as the location with the greatest variations in RH_{mean} and the only location where there was an increase in humidity in the period 1991-2020 compared to the previous climate normal. Reduction of RH_{mean}, in other locations ranges from 0.05 in Mostar to 3.64 in Zagreb.

Decrease in relative humidity of air indicates an increase in the potential for the process of evapotranspiration, i.e. faster drying of the soil and eventually more severe droughts. On the other hand, this can also be positive because in conditions of excessive rainfall, the soil will dry out sooner and can be used earlier in agriculture. For a better understanding of this parameter, an analysis at a detailed temporal (season, month) and spatial scale is needed.

Solar radiation (n) is one of the basic factors of evapotranspiration and the change of this parameter significantly affects all phases of Water cycle. Solar radiation data for this study is shown in Table 6.

Table 6. Mean solar radiation (n) and differences between analysed periods

Solar radiation (h/day)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	x̄	6.10	4.82	4.82	4.79	5.08	5.02	6.25	5.86	7.08	7.12
	σ	0.45	0.38	0.35	0.44	0.41	0.39	0.35	0.37	0.33	0.29
	CV	7.40	7.89	7.28	9.14	8.10	7.82	5.54	6.35	4.73	4.03
Current normal 1991-2020	x̄	6.60	5.42	5.19	5.35	5.57	5.54	6.64	6.33	7.38	7.30
	σ	0.46	0.49	0.37	0.48	0.40	0.42	0.38	0.45	0.39	0.37
	CV	7.04	8.98	7.04	9.00	7.16	7.67	5.66	7.16	5.30	5.02
Difference	x̄	0.50	0.60	0.36	0.56	0.49	0.52	0.40	0.47	0.29	0.18
	σ	0.01	0.11	0.01	0.04	-0.01	0.03	0.03	0.08	0.06	0.08
	CV	-0.37	1.09	-0.24	-0.14	-0.94	-0.15	0.12	0.80	0.57	1.00

The most hours of sunshine are in WS Split and Dubrovnik (more than 7.3 hours per day), and the least in Sarajevo (5.19 hours per day). This parameter shows increase at all investigated WS, ranging from 0.18 to 0.6. The biggest increase is in Sanski Most.

The increase in solar radiation is accompanied by increase in air temperature across all investigated WS. This indicates that the amount of solar radiation that reaches the earth's surface is the main cause of the increase in air temperature. The exception is Zagreb, where there is a disproportionate increase in air temperature (1.61 ºC). However, it is an urban environment, and probably the effect of urbanization in the period after 1990 contributes to the increase in temperature.

Water balance components

As shown in Table 7, an increase in ET0 was determined at all WS for the current normal climate period, most pronounced in WS Dubrovnik. The determined increase in n, T and RH also resulted in an increase in ET_0.

Table 7. Reference evapotranspiration (ET_0) and differences between analysed periods

Reference evapotranspiration (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	786	752	761	724	738	876	1065	952	1218	1128
	σ	40	43	41	39	34	39	64	42	52	59
	CV	5.15	5.78	5.41	5.33	4.65	4.40	5.98	4.40	4.28	5.27
	b	2.83	1.23	0.25	-0.52	1.68	0.09	-0.33	-0.88	-0.21	2.70
Current normal 1991-2020	$\bar{x}$	825	802	810	**752**	811	939	1103	1018	**1265**	1227
	σ	39	44	44	51	46	44	66	51	55	65
	CV	4.67	5.45	5.47	6.81	5.68	4.67	6.00	5.00	4.37	5.31
	b	-0.37	0.35	1.79	11.55	2.17	1.44	-2.58	1.74	1.38	3.16
Difference	$\bar{x}$	39	50	49	28	73	63	38	66	46	99
	σ	-2	0	3	13	12	5	3	9	3	6
	CV	-0.48	-0.33	0.06	1.49	1.03	0.28	0.02	0.60	0.09	0.04

The highest ET_0 values were determined in the south, more precisely in Split (1265 mm), followed by Dubrovnik (1227 mm) and Mostar (1103 mm). The lowest values were in Tuzla, where they amount to 752 mm per year.

The analysis of trends shows significant differences both between the periods and the locations. The highest positive trend was recorded in Tuzla, 11.55 mm/year. It is interesting to note that two locations show a small negative trend for this parameter: Livno and Mostar (from -0.37 to -2.58 mm/year).

The highest amount of snowfall is in WS Livno: 265 mm per year, while in WS Dubrovnik and Split it almost never snows and therefore could not be the subject of these calculations. From the data (Table 8), it can be seen that in the CN period in all WS locations there is a decrease in the amount of snow.

Table 8. Mean annual amount of snow and differences between analysed periods

SNOW (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	326	192	219	172	133	135	65	43		
	σ	130	84	91	69	74	56	71	54		
	CV	40	44	41	40	56	42	110	125		
	b	-2.69	-2.96	-2.63	-2.08	-2.81	-1.37	-1.87	0.21		
Current normal 1991-2020	$\bar{x}$	**265**	173	191	144	105	98	24	22		
	σ	127	82	98	79	52	49	34	28		
	CV	48	47	51	55	50	50	138	132		
	B	-0.21	-2.27	-2.58	-1.95	-2.24	-1.45	0.11	0.09		
Difference	$\bar{x}$	-61	-19	-28	-28	-29	-37	-40	-22		
	Σ	-2	-2	7	11	-22	-7	-38	-25		
	CV	8.20	3.44	9.77	15.20	-6.11	8.78	27.85	6.90		

This reduction ranges from 19 mm in Sanski Most up to 61 mm in Livno. The decrease is accompanied by a negative trend in both analyzed periods, ranging from -0.21 to -2.96 mm per year, with the exception of Mostar and Rijeka, where there is a stable trend. However, these are locations with very rare snowfall, so few years with snow were taken into account and the trend data should be taken with caution.

Similar trends were found in other studies, Čadro *et al.* (2020) reported a decreasing trend in the amount of snowfall for the area of Posavina (-0.67 to -1.66 mm/year). Similarly, the trends for snowfall ranged from -0.42 to -3.54 mm/year across the entire area of BiH (Čadro *et al.* 2019).

Large differences were found between locations in terms of mean annual runoff (Table 9). The highest value was in Rijeka (793 mm) and Mostar (684 mm), and the lowest in Osijek (124 mm). Differences between the two climatic periods indicate a decrease in RO in the southern locations, ie in Dubrovnik, Mostar, Sarajevo and Split. On the other hand, a significant increase is recorded at WS Rijeka (by 65 mm). However, by analyzing the trend, the earlier negative trend has been replaced by a positive one. This is particularly the case in Mostar, Livno and Dubrovnik.

Table 9. Mean annual runoff (blue water) in the analysed periods at 10 used weather stations

Runoff (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	500	357	301	263	118	143	745	729	172	435
	σ	158	92	113	89	62	71	258	191	79	188
	CV	32	26	38	34	52	50	35	26	46	43
	b	-6.11	-2.23	-1.67	-1.85	-2.20	-2.24	-10.28	-1.40	-3.57	-13.82
Current normal 1991-2020	$\bar{x}$	527	393	289	287	124	160	684	793	163	360
	σ	149	116	88	118	66	69	273	228	87	185
	CV	28	29	31	41	53	43	40	29	53	51
	b	5.20	0.73	-1.30	1.48	-0.95	1.74	5.76	5.21	2.32	6.36
Difference	$\bar{x}$	27	36	-12	24	6	17	-62	65	-9	-75
	σ	-9	23	-25	29	4	-2	15	37	8	-3
	CV	-3.36	3.56	-6.96	7.17	0.68	-6.38	5.29	2.51	7.13	8.08

The spring rain, which is present in this climate, often has a high intensity (Popov *et al.* 2017), therefore runoff (RO) is accelerated and the water does not stay long enough for the soil to absorb it, i.e. for the plants to use it. Under these conditions, soil erosion and flooding occur.

Table 10 shows the annual average soil moisture deficit (SMD). This parameter is often taken as an indicator of agricultural drought, or general needs for irrigation. Similar to runoff, there are large differences in SMD between the studied WS. The highest values were in Split (631 mm) and the lowest in Tuzla (103 mm). As was the case with temperatures and ET_0, the values of this parameter are higher in the CN period. This increase has a large range (12 – 101 mm), with highest increases in Dubrovnik and Rijeka, about 100 mm. The analysis of the trend (b) indicates an increase in water deficit in Tuzla and Osijek (2.4 and 2.6 mm per year), and reduction in Mostar. Such situations have already caused an increase in dry periods, and according to the obtained trend, especially in the continental part, even more negative effects can be expected.

Table 10. Mean annual soil moisture deficit (SMD) in the analysed periods at 10 used weather stations

Soil moisture deficit (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	147	88	134	91	205	168	301	125	565	369
	σ	71	62	84	78	69	89	118	73	125	131
	CV	48	70	63	85	33	53	39	58	22	36
	b	0.77	1.12	0.75	-1.31	3.24	1.49	1.74	-0.18	1.05	5.33
Current normal 1991-2020	$\bar{x}$	193	134	150	**103**	234	226	340	224	**631**	470
	σ	79	84	81	86	117	118	132	105	140	142
	CV	41	63	54	84	50	52	39	47	22	30
	b	-0.58	0.53	0.47	2.40	2.60	0.18	-2.10	0.56	0.16	-0.27
Difference	$\bar{x}$	46	45	17	12	29	59	39	99	66	101
	σ	8	22	-3	8	49	30	14	32	15	10
	CV	-7.17	-7.29	-9.13	-1.80	16.65	-0.55	-0.54	-11.62	0.04	-5.47

Table 11 shows the annual average actual evapotranspiration (AET). Given that AET represents the water that has actually evaporated and transpired, often called "green water", it can also be taken as an indicator of the plant growth and is therefore very important for analysis of the climate change impact to the region of Bosnia and Herzegovina and Croatia.

The highest values are in Rijeka (794 mm) and the lowest in Osijek (577 mm). The differences between the two climatic periods show an increase in the continental part (up to 44 mm in Osijek) and a decrease in the Mediterranean part (up to 33 mm in Rijeka). Therefore, the continental and Mediterranean zones are clearly different in regard to the impact of climate change on AET.

However, trends do not differ much, they are not uniform and do not match any temporal or spatial form. The most interesting trend is in the area of Tuzla, which shows an increase in AET of 9.15 mm per year. Also, there is an interesting change in the trend in the Mediterranean area, where in the RN period the trend was negative (from -0.70 to -2.63 mm per year), and in the current period the CN is close to zero or positive (from -0.48 to 3.43 mm per year). On the other hand, larger variations are noticeable at the WS located in the continental areas.

Table 11. Mean annual actual evapotranspiration (AET or green water) in the analysed periods at 10 used weather stations

Actual evapotranspiration (mm)		Livno	Sanski Most	Sarajevo	Tuzla	Osijek	Zagreb	Mostar	Rijeka	Split	Dubrovnik
		Continental climate						Mediterranean climate			
Reference normal 1961-1990	$\bar{x}$	640	663	627	632	533	709	764	827	653	759
	σ	69	47	64	55	41	63	86	68	91	110
	CV	11	7	10	9	8	9	11	8	14	14
	b	2.06	0.11	-0.50	0.78	-1.56	-1.39	-2.07	-0.70	-1.25	-2.63
Current normal 1991-2020	$\bar{x}$	632	668	659	649	577	713	764	794	634	757
	σ	52	54	52	67	86	89	86	76	108	109
	CV	8	8	8	10	15	13	11	10	17	14
	b	0.21	-0.18	1.32	9.15	-0.43	1.26	-0.48	1.18	1.22	3.43
Difference	$\bar{x}$	-7	5	32	17	44	4	-1	-33	-20	-2
	σ	-16	8	-12	12	45	27	0	8	17	-1
	CV	-2.47	1.10	-2.34	1.70	7.20	3.68	0.00	1.35	3.07	-0.05

CONCLUSIONS

Observing the changes that occurred between the reference climate period (1961-1990) and the current normal period (1991-2020), an increase in the mean annual air temperature (0.18–0.6 °C), solar radiation (0.18–0.6 h per day), reference evapotranspiration (28–99 mm) and soil moisture deficit (12–101 mm) was determined across all studied WS in BiH and Croatia, while the air humidity (up to 3.64 %) and amount of snow (19–61 mm) was reduced.

The differences between the continental and Mediterranean parts of the research area are evident in the amount of precipitation, runoff and actual evapotranspiration. In the north, in the continental climate, an increase in the

value of these parameters was found, while in the south, in the Mediterranean climate, a decrease was found.

The key characteristic of the current normal period compared to reference normal in both climates is the greater variation of all components of the water balance. As the result of such climate variations, the likelihood of occurrence of years with extreme rainfall in spring/autumn and extreme droughts during the summer is increased.

The reduction of snowfall in continental conditions has a whole range of negative consequences. Soils and plants are more exposed to low winter temperatures, the effect of soil erosion increases, and underground water sources are less likely to recharge.

Furthermore, the increase in soil moisture deficit and solar radiation, as well as decrease of air humidity, especially in conditions of dry spring or extreme rains increase drought severity, and the availability of water for all sectors can become an issue.

It is very important to carry out these analyzes in detail, at the level of seasons and even months in order to better understand the impact of these changes throughout the year, especially from the aspect of agriculture and the period when plants have the highest water requirements (vegetation period). Also, it is very important to determine the “urban heat island” effect (UHI) in order to reduce the effect of urbanization to a minimum and obtain the most accurate data for a wider area, not just the urban environment. In the future, with the development of remote sensing observation technologies, primarily satellites and local corrections with measurements from the ground, this problem will surely be solved.

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PHENOTYPIC CHARACTERIZATION OF THE ACCESSIONS OF FABA BEAN (VICIA FABA L.) STORED IN THE GENETIC BANK OF ALBANIA

SUMMARY

The study was done to determine the variation of 12 morphological and agronomic traits in 17 landraces of faba bean (*Vicia faba*), collected in areas of Albania and stored in the Genetic Bank of Albania, to use them for cultivation purposes or in genetic improvement programs in function of agricultural production. For the purpose of this study, 12 morphological and agronomic traits were analyzed: leaf length, leaf width, plant height, number of branches on the main stem, number of pods per plant, number of kernels per pod, number of kernels per plant, pod length, pod width, kernel length, kernel width and 100 kernel weight. Validated differences were found for all 12 morphological and agronomic traits. The landraces of faba bean are valued as material for the cultivation of faba bean for the production of legumes and grains. We consider that the large load of the plant with fruit organs can affect the reduction of the weight of 100 kernels.

Key words: faba bean, landrace, phenotypic variation, *Vicia faba*

INTRODUCTION

Faba bean (*Vicia faba* L.) is a winter leguminous crop originating in the Middle East in prehistoric times, belonging to the genus *Vicia* and the Family Leguminosae (*Fabaceae*) and traditionally used as a main source of protein for human and animal nutrition (Multari *et al*., 2015). The leguminosae family

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(*Fabaceae*), in terms of economic importance, is the second family, only after the grasses (*Poaceae*).

Under optimal cultivation conditions, the germination of faba bean seeds takes 10–14 days (Etemadi *et al*., 2015). However, it will take much longer to germinate in dry conditions or when the soil is cold. On average, the faba bean plant grows one node per week. As the stem of the faba bean is relatively strong and grows vertically, the plant can grow to a height of 90–130 cm, depending on the genotype. At the growth stage of nodes 8–10, when the plant is about 30 cm high, the faba bean forms the first flowers. The flowers and pods appear about 20 cm above the ground. Approximately 25% of the flowers will set pods, which usually contain three to six seeds (Etemadi *et al*., 2015). Therefore, the application of appropriate cultivation technology, including irrigation, soil fertility and planting time can significantly reduce the number of aborted flowers, thus improving the final yield of grains/pods.

In general, seed germination of most legumes is sensitive to low soil temperature. However, faba bean is one of the few winter grain legumes whose germination can tolerate cold soil temperatures better than most legumes. Attempts to select for improved seed germination at soil temperatures below 15°C have shown success rates (Singh and Jauhar, 2005). Better germination rate at 12.5°C has been reported in large-seeded cultivars than in small-seeded cultivars (Kang *et al*., 2008).

Due to superior nutritional values including proteins, carbohydrates, B group vitamins and minerals (Crépon *et al.,* 2010), faba bean is considered one of the most important leguminous crops in the world. As a winter legume, faba bean can be incorporated into various farming systems.

Faba bean is partially self-pollinating plant, however the flowers attract various pollinators, especially bees. Current reports showed that bees and other natural pollinators can increase the pollination rate and therefore the grain yield of ryegrass (Musallam *et al*., 2004, Marzinzig *et al*., 2018).

Legumes are a sustainable source of high protein food and are widely cultivated throughout the world. Among legumes, faba bean (*Vicia faba* L.) is one of the oldest agricultural crops cultivated worldwide (Mínguez & Rubiales, 2021). Faba bean is considered an ecologically, nutritionally and economically important agricultural crop (Xiao *et al*., 2021). It is a multipurpose plant that provides various ecosystem services, that is, it is cultivated mainly as a source of food for humans living in Asia and Africa, as food for humans and/or animals in the European region, and for fixing atmospheric nitrogen in agricultural soils by significantly reducing the use of chemical fertilizers (Zhou *et al*., 2018). Nutritionally, mature grain faba bean are rich in protein (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%), (USDA, 2021). Faba bean also contains bioactive compounds, for example, total phenolics and flavonoids with demonstrated antioxidant activity (Valente *et al*., 2018). Faba bean consumption can cause the condition known as favism – a severe form of hemolytic anemia

(Mínguez & Rubiales, 2021; Singh *et al.,* 2013). Hulse (1994) reported that the concentration of lectins (hemagglutinins) is higher in faba bean than in other legumes. Also, oligosaccharides (stachyose, raffinose and verbascose) are also present in faba beans (Toklu *et al.,* 2021), which can be fermented and produce flatulence, which leads to abdominal discomfort. Lectins are destroyed during normal cooking processes due to high heat.

Faba bean has been identified to have the ability to efficiently fix N, and this ability is the highest among winter legumes (Mekkei, 2014). Reports indicate that faba bean can fix 50–330 kg N/hm (Galloway *et al*., 2004, Etemadi *et al.,* 2018) depending on cultivation technology and environmental conditions (Hu and Schmidhalter, 2005). Legumes have an essential role in maintaining soil fertility, not only through biological N fixation, but also by dissolving insoluble phosphorus (P) in the soil, improving the soil's physical environment and increasing soil microbial activity (Rashid *et al.* , 2016).

Genetic variation for N fixation has been identified in faba bean (Graham and Vance, 2000). It is well known that legumes generally respond to existing soil N concentration (Graham, 2008). In soils with relatively high N content, legumes use more N that is in the soil instead of engaging in symbiosis with rhizobia to fix air N (Divito and Sadras, 2014). But the use of N at minimal rates can stimulate N fixation by improving the early stages of plant cultivation until N fixation provides adequate N for plant growth and development (Huang *et al.,* 2017, Abdul Rahman *et al*., 2018).

Faba bean is one of the main leguminous crops that is cultivated in Albania mainly on land above water. In Albania, the farmer's cultivars (landraces) of faba bean have not been genetically evaluated. Characterization of the genetic variation of available faba bean germplasm is important for further improvement of faba bean yield and resistance to biotic and abiotic stresses. In Albania, faba bean grain can potentially be an important alternative source of protein in the feeding of non-ruminant and ruminant animals (Dalipaj, 2021). However, faba bean grain also contains some secondary metabolites, such as phenolic compounds, which for years have been considered as antinutrients, but some later studies have shown their positive effects in the animal organism, considering them as micronutrients (Dalipaj, 2021). Interest in identifying and quantifying the phenolic compounds present in faba bean has increased based on their positive biological effects on health (Dalipaj, 2021). Moreover, the antioxidant activity of phenolic compounds has the property to prevent some diseases and improve the productive performance of animals (Dalipaj, 2021). The kernel of faba bean has lower protein, methionine and cysteine content than soybean, but higher lysine and starch content (Dalipaj, 2021) and higher ruminal degradation of protein (Dalipaj, 2021). Several studies have shown the positive effects of including kernel faba bean in the diet of cows and sheep (Dalipaj, 2021). Its nutritional values have shown variation more as a result of the cultivar than from the methods of their cultivation (Dalipaj, 2021).

The world production of faba bean in 2019 was 5.43 million tons, which represented about 25% increase compared to 4.35 million tons in 1990. The more than 50 countries producing faba bean, about 90% of the production is concentrated in the Asia region with 33.55% of the total world production, followed by the countries of the European Union, with 29.36%, and from African countries with 27.04% of world production (FAO, 2020). Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe and North Africa, are the main producers of faba bean (Rahate *et al.,* 2020). China has been the leading producer of faba bean, followed by Ethiopia (Barri and Shtaya, 2012); these two countries represented about 50% of total world production, while the countries of the European Union, the United Kingdom and France were among the top five producers. Also, in 2019, Australia was the leading exporter of faba bean with 265,543 tones or nearly 30% of total exports, followed by the United Kingdom, Lithuania, Egypt and Latvia (FAO, 2020). Egypt leads the importers with 309,355 tons or 40.48% of world imports, followed by Norway, Germany, Saudi Arabia and France (FAO, 2020)

The aim of the study was to know the characteristics of the accessions of the Albanian faba beans. By determining the values of 12 morphological characteristics in 17 accessions of faba beans (*Vicia faba*), collected in the areas of Albania and stored in the National Genetic Bank, to evaluate their morphological and agronomic variation in order to use them for cultivation purposes or and in genetic improvement programs in function of agricultural production.

MATERIAL AND METHODS

Plant materials used in the study

In the study, 17 accessions of faba bean (*Vicia faba*), collected in areas of Albania and stored in the National Genetic Bank, were taken, as follows: 1) AGB2476, 2) AGB2477, 3) AGB2478, 4) AGB2479, 5) AGB2480, 6) AGB2481, 7) AGB2482, 8) AGB2483, 9) AGB2484, 10) AGB2485, 11) AGB2486, 12) AGB2487, 13) AGB2488, 14) AGB2489, 15) AGB2490, 16) AGB2491, 17) AGB2492.

The study was conducted in the Didactic Experimental Economy of Agriculture of the University of Tirana (latitude: 402405N; longitude: 0194108E; altitude of 40m). The study was conducted in two agricultural years, 2018–2019 and 2019–2020. The design of the experiment was a randomized complete block with four replications. All accessions were planted on November 5, 2018 and November 10, 2019. Each variety was planted in 5 rows of 3 m length and 60 cm row spacing; the seed was planted at a distance of 20 cm from each other, in each replication. So, each variant was represented with an area of 10.5 m^2 in every replication. For each accession, 30 seeds were planted in a row. After planting, the plot was immediately irrigated. Then, during the vegetation, all the necessary agrotechnical services of the faba bean were performed.

Obtained data of morphological characteristics:

During the vegetation and until the ripening of the pods, the data were recorded for the purpose of the study. At the stage of full pod development (green pods) the following data were obtained on 5 plants in the middle of the row from each variant for all four replicates.

1.Leaflet length, cm; measured in the middle of the leaflet;

2.Leaflet width, cm; measured in the length of the leaflet;

3.Plant height, cm; measured at near maturity from ground surface to the tip of the plant;

4.Number of branches/plant; recorded as total number of branches from the basal node;

5.Number of pods per plant; recorded as the average of five plants;

6.Number of kernels/pod; was counted for ten random pods;

7.Number of kernels/plant; was number of pods/plant x number of kernels/pod;

8.Pod length, cm; for five random pods, measured as the distance between the edges and pod;

9.Pod width, cm; measured for five random pods at the center of pod using a caliper;

10.Kernel length, cm; measured for five random kernels, using a caliper;

11.Kernel width, cm; measured for five random kernels, using a caliper;

12.100 kernels weight, g; determined by mixing the whole samples, then 100 kernels were randomly counted and weighted.

Field observations

Field observations of the experiment and various measurements were based on the standards of the Genetic Bank (FAO, 2014), Recognized Reconstruction Guidelines (Salcedo, 2008) and Faba Bean's Descriptors (IBPGR /85/116 June 1985).

Statistical analysis

All the experiment data were subjected to analysis of variance to know the validity of the experiment data. In order to find any correlation between the traits under study, correlation coefficients were also calculated. The evaluation of the correlations was done according to this order: r = ±3, the correlation is weak; ±3 to ±5, correlation is medium; ±5 to ±7, correlation is good; ±7 to ±9, the correlation is strong. Finally, cluster analysis was done using the full linkage method.

RESULTS AND DISCUSSION

The data obtained from the comparative field trial of 17 accessions of faba bean landraces for their 12 field characteristics were first subjected to analysis of variance to see if the experimental data are validated. According to the data of this analysis, the data of all 12 morphological and agronomic characteristics for the two years of the study (2018–2019 and 2019–2020) the differences between

the variants (accessions) are confirmed for the level of probability P≤0.01, which will show that the differences between the accessions are statistically proven, that is, the differences between the accessions are controlled by genetic factors. For some morphological and agronomic traits, differences between replicates are also established. Thus, for example, for the year 2018-2019, differences are confirmed for 7 traits, while for the year 2019-2020, differences are confirmed for 10 traits, which shows that these traits, in addition to genetic factors, are also influenced by environmental factors. Based on the results of this analysis, let's further examine the data of the study.

1. Leaflet length

Even the variation of leaflet length in 2019 was relatively narrow (table no. 1), 6 accessions (35.3%) were in the first group with 7.39a–6.40 cm and 11 accessions (64,7 %) had the smallest leaflet length (5.66–6.29 cm). In 2020, the variation for leaflet length was more pronounced, all 17 accessions were divided into 4 groups; accession AGB2482 had the highest value (10.51a cm), while accession AGB2489 had the lowest value, with 9.01de cm. However, the greatest average value of leaflet length was in 2020, with 9.49 cm, compared to 2019, which was 6.21 cm.

2.Leaflet width

The variation for leaflet width in 2019 was good (table no. 1) where 3 accessions (AGB2484, AGB2481 and AGB2487) had the widest leaf, respectively 3.37a cm, 3.18a cm and 3.09ab cm. In this year, the narrowest leaflet was recorded in accessions AGB2477, AGB2486 and AGB2485, respectively with 2.15df cm, 2.34de cm and 2.23de cm. In 2020, the average leaflet width was greater (4.68 cm), compared to 2019 (2.66 cm), but the variation of its width was also wider (table no. 3). Interestingly, only one accession (AGB2478) entered the first group with the widest leaflet (5.62a cm), 3 accessions (AGB2476, AGB2486 and AGB2488) had the narrowest leaflet, respectively 4.31gi cm, 4, 34gi cm and 4.36gi cm.

3.Plant height

For plant height, the highest average value was in 2020, with 96.83 cm, from 64.31 cm in 2019 (table no. 1). For both years of the study, there was good variation for this trait. In 2019, there were two accessions (AGB2479 and AGB2483) with the highest plant height, respectively 79.36a cm and 76.81a cm, while the shortest plant was accession AGB2492 with 48.94gi cm. In 2020, accession AGB2476 grew the tallest plant with 111.1a cm, while the shortest plant was accession AGB2485 with 87.00gi cm. Since the plants were taller in 2020, it appears that conditions that year were better for this plant.

Table no. 1: Average values of leaflet length, leaflet width and plant height, 2019 and 2020

Accession	df		Values of morphological and agronomic traits					
			Leaflet length, cm		Leaflet width, cm		Plant height, cm	
	Treatments	Replications	2019	2020	2019	2020	2019	2020
AGB2476	16	3	5,90**b**	9,64**bc**	2,52**cd**	4,31**gi**	70,17**bc**	111,19**a**
AGB2477	16	3	6,00**b**	9,29**cd**	2,15**df**	4,56**fg**	62,67**de**	94,04**ef**
AGB2478	16	3	6,29**b**	9,56**c**	2,72**bc**	5,62**a**	58,16**ef**	101,39**cd**
AGB2479	16	3	5,66**bc**	9,99**b**	2,43**cd**	4,58**fg**	79,36**a**	89,76**fh**
AGB2480	16	3	6,53**ab**	9,35**cd**	2,76**bc**	5,18**c**	64,98**de**	105,48**b**
AGB2481	16	3	5,74**bc**	9,56**c**	3,18**a**	4,68**eg**	60,71**df**	95,44**ef**
AGB2482	16	3	6,59**a**	10,51**a**	2,63**cd**	4,46**fh**	66,57**cd**	95,62**ef**
AGB2483	16	3	6,00**b**	9,85**bc**	2,68**c**	4,44**fh**	76,81**a**	94,43**ef**
AGB2484	16	3	7,39**a**	9,52**cd**	3,37**a**	4,24**gj**	66,04**cd**	95,62**ef**
AGB2485	16	3	6,94**a**	9,37**cd**	2,24**de**	4,78**ef**	60,48**df**	87,00**gi**
AGB2486	16	3	6,17**b**	9,25**cd**	2,23**de**	4,34**gi**	66,08**cd**	94,78**ef**
AGB2487	16	3	5,82**b**	9,16**de**	3,09**ab**	4,69**eg**	66,07**cd**	104,09**bc**
AGB2488	16	3	5,94b	9,22**cd**	2,96**b**	4,36**gi**	52,79**fh**	93,07**eg**
AGB2489	16	3	6,60**a**	9,01**de**	2,86**bc**	4,69**eg**	64,15**de**	100,90**cd**
AGB2490	16	3	5,95**b**	9,21**cd**	2,55**cd**	4,57**fg**	64,02**de**	94,87**ef**
AGB2491	16	3	6,40**ab**	9,23**cd**	2,41**cd**	4,64**eg**	65,32**cd**	94,58**ef**
AGB2492	16	3	5,72**bc**	9,55**c**	2,48**cd**	5,40**b**	48,94**gi**	93,85**ef**
Average			**6,21**	**9,49**	**2,66**	**4,68**	**64,31**	**96,83**
D_{01} / D_{05}			**1,07/0,8**	**0,44/0,33**	**0,32/0,24**	**0,20/0,15**	**4,75/3,56**	**3,87/2,90**

4.Number of branches per plant

In 2019, the variation in the number of branches on the main stem was weak; all 17 accessions were classified in only two groups (table no. 2).

Table no. 2: Average values of the branches per plant, pods per plant and of the kernels per pod, 2019 and 2020

Accession	df		Values of morphological and agronomic traits					
			Branches/plant		Pods/plant		Kernels/pod	
	Treatments	Replications	2019	2020	2019	2020	2019	2020
AGB2476	16	3	3,4**b**	5,6**de**	22,03**a**	24,8**cd**	5,93**b**	8,73**b**
AGB2477	16	3	3,1**bc**	5,4**ef**	16,44**v}**	15,6**hj**	6,59**a**	8,49**b**
AGB2478	16	3	3,0**bc**	4,7**fh**	17,50**rw**	21,6**ef**	5,56**b**	8,49**b**
AGB2479	16	3	3,4**ab**	4,8**fh**	12,49	25,9**cd**	5,60**b**	7,97**bc**
AGB2480	16	3	2,8**bc**	5,6**de**	18,46**nr**	25,3**cd**	6,02**ab**	8,29**b**
AGB2481	16	3	3,4**b**	4,4**gi**	15,43**y**	25,9**c**	5,68**b**	8,67**b**
AGB2482	16	3	3,4**b**	6,3**c**	14,37**}**	18,3**fh**	5,93**b**	8,16**b**
AGB2483	16	3	3,1**b**	5,6**de**	14,22~	22,4**df**	5,66**b**	7,36**bc**
AGB2484	16	3	4,1**a**	4,4**gi**	17,20**sy**	26,0**c**	4,85**bc**	8,34**b**
AGB2485	16	3	3,6**a**	6,1**cd**	17,28**rx**	23,1**de**	5,90**b**	8,17**b**
AGB2486	16	3	3,1**bc**	7,2**a**	15,05**{**	24,8**cd**	5,84**b**	7,66**bc**
AGB2487	16	3	3,3**b**	6,3**c**	15,44**y**	30,7**a**	5,87**b**	10,92**a**
AGB2488	16	3	2,9**bc**	5,8**de**	14,84|	20,7**eg**	6,14**ab**	8,50**b**
AGB2489	16	3	3,0**b**	4,6**gi**	14,60|	24,1**de**	6,62**a**	10,33**a**
AGB2490	16	3	2,9**bc**	4,7**fh**	17,04**sz**	15,8**gj**	7,86**a**	9,96**a**
AGB2491	16	3	3,6**a**	3,5**il**	14,44**}**	14,6**hk**	7,45**a**	9,54**ab**
AGB2492	16	3	3,4**b**	5,0**fg**	14,36**}**	23,6**de**	7,82**a**	11,64**a**
Average			**3,3**	**5,3**	**15,95**	**22,5**	**6,19**	**8,89**
D_{01} / D_{05}			**0,7/0,5**	**0,42/0.32**	**0,27/0,20**	**2,1/1,6**	**1,86/1,39**	**2,33/1,75**

Four accessions (AGB2484, AGB2485, AGB2491 and AGB2479) had the largest number of branches in this year with, respectively, 4.1a; 3.6a, 3.6a and

3.4ab branches per plant, while accessions AGB2480, AGB2488 and AGB2490 had the smallest number of branches with, respectively, 2.8bd, 2.9bc and 2.9bc branches. In 2020, the variation in the number of branches was considerable; all 17 accessions for this trait were classified into 7 groups. In this year, accession AGB2486 stood out for the largest number of branches on the main stem with 7.2 branches, while accession AGB2491 stood out for the smallest number of branches with 3.5 branches. By comparing the data of this trait between the two years of the study, we noticed that in 2020 the faba bean plants created an average of more branches (5.3) compared to 2019 when the plants had an average of 3.3 branches.

5.Number of pods per plant

The number of pods per plant, as a production trait, is important in the evaluation of planting material. In the analysis of this study, it is of interest that this feature is represented by the considerable variation for the faba bean accessions taken in the study. In 2019, accession AGB2476 had the highest number of pods per plant (table no. 2), which tied and developed 22.03a pods, while accession AGB2479 had the lowest number of pods per plant, which averaged only 12.49 pods. In 2020, accession AGB2487 had the largest number of pods per plant with 30.7 ha of pods, while accessions AGB2491 with 14.6 ha of pods, AGB2477 with 15.6 ha and AGB2490 with 15.8 ha had the lowest number of pods. The average number of pods per plant was reached in 2020 (22.5) compared to 2019 with 15.95 pods.

6.Number of kernels per pod

The number of kernels per pod was presented with weak variation in both years of the study (table no. 2). In 2019, the highest number of kernels per pod appeared in 6 accessions, out of 17 accessions in the study, which had from 7.86a to 6.14ab kernels per pod, while the lowest number of kernels per pod was had accession AGB2484 which had bound 4.85bc kernels per pod. In 2020, five accessions (AGB2492, AGB2487, AGB2489, AGB2490 and AGB2491) were distinguished for the highest number of kernels per pod, which they developed, respectively from 11.64a; 10.92a; 10.33a, 9.96a and 9.54ab kernels per pod. Whereas three accessions (AGB2483, AGB2486 and AGB2479) had the smallest number, which had, respectively, from 7.36bc; 7.66bc and 7.97bc kernels per pod. The highest average number of kernels per pod was in 2020 with 8.89 kernels, compared to 2019 with 6.19 kernels per pod.

7.Kernels per plant

The values of the number of kernels per plant had a relatively weak variation, but in 2020 somewhat better than in 2019 (table no. 3). In 2019, six accessions (AGB2490, AGB2476, AGB2492, AGB2480, AGB2477 and AGB2491) stood out for the highest number of kernels per plant with, respectively, 133.88a; 130,61a; 112,15ab; 111.09ab; 108.28ab and 107.59ab kernels per plant. As for the smallest number of kernels per plant, the accession AGB2479 appeared with only 66.93c kernels. In 2020 there was a higher number of kernels per plant for all accessions. But, for the largest number of kernels in

2020, the accessions AGB2487 with 334.79 kernels and AGB2492 with 274.69 a kernels per plant stood out and, for the smallest number of kernels per plant, the accessions AGB2477, AGB2491 and AGB2482 with, respectively, 132.15de; 139.03 and 149.10 kernels per plant. The average number of kernels per plant was in 2020 with 200.35 kernels compared to 2019 with 98.54 kernels per plant.

8.Pod length

For the length of the pod, there is satisfactory variation in the data of 2019 and somewhat poor in 2020 (table no. 3).

Table no. 3: Average values of the kernels per plant, of the pod length and of the pod width, 2019 and 2020

Accession	df		Values of morphological and agronomic traits					
			Kernels/plant		Pod length		Pod width	
	Treatments	Replications	2019	2020	2019	2020	2019	2020
AGB2476	16	3	130,61**a**	216,25**bc**	8,76**cd**	10,22**cd**	1,29**b**	1,45**bc**
AGB2477	16	3	108,28**ab**	132,15**de**	10,96**a**	9,75**cd**	1,16**bc**	1,68**ab**
AGB2478	16	3	97,22**b**	183,64**cd**	9,60**bc**	11,07**bc**	1,26**b**	1,63**b**
AGB2479	16	3	69,93**c**	205,03**c**	9,96**b**	9,92**cd**	1,47**a**	1,73**ab**
AGB2480	16	3	111,09**ab**	208,52**c**	8,85**cd**	10,28**cd**	1,23**b**	1,35**bc**
AGB2481	16	3	87,65**bc**	223,83**bc**	9,31**bc**	11,21**bc**	1,26**b**	1,38**bc**
AGB2482	16	3	85,02**bc**	149,10**de**	9,60**bc**	11,54**b**	1,56**a**	1,66**ab**
AGB2483	16	3	80,38**bc**	164,60**cd**	9,33**bc**	9,90**cd**	1,17**bc**	1,40**bc**
AGB2484	16	3	8342**bc**	217,07**bc**	10,95**a**	12,97**a**	1,49**a**	2,06**a**
AGB2485	16	3	101,79**b**	187,66**cd**	8,46**cd**	10,68**bc**	1,05**c**	1,34**bc**
AGB2486	16	3	87,95**bc**	189,21**cd**	6,94**eg**	9,22**de**	1,17**bc**	1,58**b**
AGB2487	16	3	90,58**b**	334,79**a**	6,56**fg**	9,21**de**	1,54**a**	1,61**b**
AGB2488	16	3	91,01**b**	175,32**cd**	7,44**ef**	9,44**de**	1,33**ab**	1,49**b**
AGB2489	16	3	96,58**b**	248,17**b**	6,53**fg**	10,60**c**	1,44**a**	1,58**b**
AGB2490	16	3	133,88**a**	156,90**cd**	8,21**de**	9,41**de**	1,45**a**	1,48**b**
AGB2491	16	3	107,59**ab**	139,03**de**	7,62**df**	9,27**de**	1,35**ab**	1,40**bc**
AGB2492	16	3	112,15**ab**	274,69**ab**	9,38**bc**	9,97**cd**	1,44**a**	1,55**b**
Average			**98,54**	**200,35**	**8,73**	**10,27**	**1,33**	**1,55**
D_{01} / D_{05}			**21,7/28,9**	**61,1/45,8**	**0,86/0,64**	**1,16/087**	**0,24/0,18**	**0,40/0,30**

From the 2019 data, accessions AGB2477 and AGB2484 stood out for longest pods with, respectively, 10.96a and 10.95a cm. For shorter pods this year, accessions AGB2489, AGB2487 and AGB2486 stood out with, respectively, 6.53fg; 6.56fg and 6.94eg cm. For the year 2020, the accession AGB2484 stands out for the longest pod with 12.97a cm, while for the shortest pod there are five accessions (AGB2487, AGB2486, AGB2491, AGB2490 and AGB2488) with, respectively, 9.21de; 9.22 de; 9.27 de; 9.41 and 9.44 cm. Average pod length was greater in 2020 (10.27 cm) compared to 2019 (8.73 cm).

9.Pod width

Even for the width of the pod, the data show weak variation for both years (table no. 3). From the 2019 data we note that 9 accessions are represented by the pod with the largest width, which fluctuates between 1.56a cm and 1.33ab cm; the other 7 accessions fall into the narrower beans group, with values ranging from 1.16bc cm to 1.29b cm. In 2020, four accessions (AGB2484 with 2.06a cm;

AGB2479 with 1.73ab cm; AGB2477 with 1.68ab cm and AGB2482 with 1.66ab cm) were distinguished for the largest pod width. For the smallest pod width for this year, 13 other accessions were distinguished, with values from 1.34bc cm to 1.63b cm. Average pod width was greater in 2020 (1.55 cm), compared to 2019 (1.33 cm).

10.Kernel length

For kernel length, the data of 2019 showed higher variation than in 2020 (table no. 4). Based on the 2019 data, we note that only the accession AGB2484 has the highest kernel length value (2.31a cm). Whereas the lowest value of kernel length has the accession AGB2489 (1.37jn cm). From the 2020 data, there are fewer accession groups for this trait, therefore, 10 accessions are in the first group with the largest kernel length (table no. 4) with values from 1.88a cm to 1, 65ab cm, the other six accessions have the smallest kernel length, from 1.41c cm to 1.62b cm. Interestingly, the average kernel length value is lower in 2020 (1.66 cm) compared to 2019, which was 1.76 cm.

11. Kernel width

In these positions are also the kernel width values, where the data of 2019 present a greater variation compared to those of 2020 (table no. 4). In 2019, only one accession stands out for the largest kernel width; accession AGB2489 with 1.96a cm, while for the smallest kernel width, accession AGB2485 stands out with a grain width of 1.25pv cm. For the year 2020, accession AGB2484 stands out for the largest kernel width with a value of 1.53a cm, while for the smallest kernel width, accession AGB2486 stands out with a kernel width of 0.95ef cm. Even for kernel width, the average value of 2020 is smaller (1.22 cm) compared to the average value of 2019 (1.52 cm).

12. 100 kernels weight

Table no. 4: Average values of the kernel length, kernel width and of the 100 kernels weight, 2019 and 2020

Accession	df		Values of morphological and agronomic traits					
			Kernel length		Kernel width		100 kernels weight	
	Treatments	Replications	2019	2020	2019	2020	2019	2020
AGB2476	16	3	1,75**gi**	1,62**b**	1,37**nr**	1,25**bc**	122,8**ik**	115,8**a**
AGB2477	16	3	1,77**fh**	1,83**a**	1,42**lp**	1,24**c**	113,3**pu**	107,0**b**
AGB2478	16	3	1,80**fh**	1,65**ab**	1,63**hj**	1,25**bc**	111,3**qw**	109,8**ab**
AGB2479	16	3	1,80**fh**	1,71**a**	1,38**mr**	1,27**bc**	116,0**nr**	99,5**cd**
AGB2480	16	3	1,77**fh**	1,53**b**	1,46**ko**	1,17**cd**	133,5**a**	95,8**de**
AGB2481	16	3	1,83**fg**	1,76**a**	1,45**lp**	1,27**bc**	116,7**mq**	113,8**a**
AGB2482	16	3	1,77**fh**	1,65**ab**	1,55**il**	1,28**bc**	127,8**ef**	99,8**cd**
AGB2483	16	3	1,76**fh**	1,50**bc**	1,36**nr**	1,20**cd**	126,8**fg**	105,9**bc**
AGB2484	16	3	2,31**a**	1,88**a**	1,84**cd**	1,53**a**	121,0**jm**	112,1**a**
AGB2485	16	3	1,66**gj**	1,72**a**	1,25**pv**	1,26**bc**	121,2**jm**	88,9**ef**
AGB2486	16	3	1,61**hj**	1,41**c**	1,63**hj**	0,95**ef**	128,8**de**	91,0**df**
AGB2487	16	3	1,74**gi**	1,57**b**	1,33**ns**	0,99**df**	126,8**fg**	88,7**ef**
AGB2488	16	3	1,66**gj**	1,59**b**	1,75**eg**	1,05**de**	124,5**gj**	97,8**cd**
AGB2489	16	3	1,37**jn**	1,71**a**	1,96**a**	1,23**c**	132,2**b**	93,3**de**
AGB2490	16	3	1,80**fh**	1,55**b**	1,76**ef**	1,19**cd**	109,0**sy**	89,7**ef**
AGB2491	16	3	1,65**hj**	1,72**a**	1,45**lp**	1,30**bc**	117,3**mq**	97,8**cd**
AGB2492	16	3	1,90**ef**	1,81**a**	1,34**ns**	1,29**bc**	97,6|	98,4**cd**
Average			**1,76**	**1,66**	**1,52**	**1,22**	**120,4**	**100,3**
D_{01} / D_{05}			**0,09/0,07**	**0,23/0,17**	**0,05/0,03**	**0,14/0,11**	**1,3/1,0**	**6,2/4,7**

The values of the weight of 100 kernels give rich variation for both years (table no. 4). In 2019, accession AGB2480 had the largest weight of 100 kernels (133.5a grams), while accession AGB2478 had the smallest weight (111.3qw grams). In 2020 there were four accessions with the largest 100-grain weight (AGB2476, AGB2481, AGB2484 and AGB2478) with corresponding 100-kernels weights of 115.8a grams; 113.8a grams, 112.1a grams and 109.8ab grams; while for the lowest weight, the accessions AGB2487, AGB2485 and AGB2490 were distinguished with the respective weights of 100 kernels 88.7ef grams, 88.9ef grams and 89.7ef grams. Again, after the length of the kernel and the width of the kernel, even for the weight of 100 grains, the value of the average weight of 100 kernels for the year 2020 is lower (100.3 grams) than that of the year 2019 (120.4 grams).

From the discussion of data on morphological and agronomic traits, it is noticeable that these traits are controlled by genetic factors but also influenced by environmental factors. This conclusion is confirmed by many studies, including two studies in Albania (Hobdari *et al*. 2019 and Gixhari *et al*. 2019).

Correlation between morphological and agronomic traits under study

The Pearson correlation coefficients between the studied characteristics were calculated separately for the data of 2019 and the data of 2020. From the data of 2019 we find four positive correlations (table no. 5), three of which are well connected:branches/plant: kernel length ($r_{02/08}$=0.53*), pod/plant:kernel/plant ($r_{03/05}$=0.66**) and kernel/pod:kernel/plant ($r_{04/05}$=0.65**), as well as a strong relationship, pod length:kernel length ($r_{06/08}$=0.72**). The data of 2020 give another panorama on the correlations (table no. 6); in them we find 8 positive correlations, of which: a medium link pod length:pod width ($r_{06/07}$=0.50*), four good links: kernel/pod:kernel/plant ($r_{04/05}$=0.61**), pod length:kernel length ($r_{06/08}$=0.53*), pod length: g/100 kernel ($r_{06/10}$=0.52*) and kernel width: g/100 kernels ($r_{09/10}$=0.55*) and three correlations of strength: pod/plant:kernel/plant ($r_{03/05}$=0.83**), pod length:kernel width ($r_{06/09}$=0.75**) and kernel length:kernel width ($r_{08/09}$=0.78**). According to these results, pods per plant, kernels per pod, kernels per plant, pod length, pod width, kernel length, kernel width and 100 kernel weight are important.

Despite the obtained correlations, the reduction of kernel indicators (kernel length, kernel width and weight of 100 kernels) in 2020 compared to 2019 is observed, while almost all morphological and agronomic traits had higher values in 2020. This fact gives us the right to judge that the greater load of the plant in 2020 with fruit organs (pod/plant, kernel/pod, kernel/plant) has influenced the smaller weight of the kernel, which may also be the result of competition for nutrients, this may be more pronounced than the number of kernel/pods, where the competition for nutrients is greater. Correlations between morphological and agronomic traits are also influenced by environmental conditions; therefore the relationships between different traits are not the same between the two years of the study. This judgment is also supported by other studies (Dollotovskij *et al*. 1989; Hyso and Kashta 2000), regardless of the agricultural crops with which

they worked. The reason why the present correlations did not reveal such relationships could be the small number of accessions as well as the non-significant differences in the number of grains per pod in the study accessions.

Table no. 5: Correlations for 10 morphological and agronomic traits of *Vicia faba* for 2019

Traits	Plant height	Branch/ plant	Pods/ plant	Kernels/ pod	Kernels/ plant	Pod length	Pod width	Kernel length	Kernel width
	1	2	3	4	5	6	7	8	9
2.Branch/plant	0,15								
3. Pods/plant	–0,07	–0,03							
4. Kernels/pod	–0,40	–0,29	–0,13						
5. Kernels/plant	–0,32	–0,28	0,66**	0,65**					
6. Pod length	0,12	0,33	0,12	–0,27	–0,12				
7. Pod width	0,06	0,24	–0,30	0,15	–0,12	–0,07			
8. Kernel length	0,01	0,53*	0,18	–0,31	–0,11	0,72**	0,24		
9. Kernel width	–0,16	–0,12	–0,05	–0,01	–0,04	–0,19	0,33	–0,02	
10.g/100kernels	0,42	–0,12	0,07	–0,51	–0,32	–0,41	–0,09	–0,37	0,21

Table no. 6: Correlations for 10 morphological and agronomic traits of *Vicia faba* for 2020

Traits	Plant height	Branch/ plant	Pods/ plant	Kernels/ pod	Kernels/ plant	Pod length	Pod width	Kernel length	Kernel width
	1	2	3	4	5	6	7	8	9
2. Branch/plant	0,05								
3. Pods/plant	0,33	0,27							
4. Kernels/pod	0,21	–0,29	0,07						
5. Kernels/plant	0,38	0,09	0,83**	0,61**					
6. Pod length	0,01	–0,23	0,20	–0,24	0,01				
7. Pod width	–0,08	–0,09	0,14	–0,03	0,11	0,50*			
8. Kernel length	–0,27	–0,58	–0,11	0,26	0,06	0,53*	0,44		
9. Kernel width	–0,15	–0,65	–0,18	–0,04	–0,19	0,75**	0,40	0,78**	
10.g/100kernels	0,27	–0,35	0,02	–0,32	–0,16	0,52*	0,24	0,40	0,55*

CONCLUSIONS

Based on the data of the study, as well as on their review and discussion, we can draw some conclusions, among which we mention:

- Landraces of fava bean are an important genetic source for science and for Albanian agriculture;
- The landraces in the study have marked differences for all morphological and agronomic features;
- The morphological and agronomic traits in the study are controlled by genetic factors, but are also influenced by environmental factors;
- The large load of the plant with fruit organs is most likely to affect the smaller weight of the kernel;
- Correlations are influenced by environmental factors, so they do not provide the same data in every environment;
- Faba bean landraces must be preserved so that they are not subject to genetic erosion.

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ANALYSIS OF FINANCIAL REPORTS OF PE "NP FRUŠKA GORA"

SUMMARY

Financial reports are a value presentation of the impact of those changes on the financial structure, business success and changes in cash flows of a company. The aim of the research is the financial analysis of the operations of the public enterprise (PE) "NP Fruška Gora". The purpose of the research is to identify problems in business and indicate opportunities for improving the economic efficiency of the business of PE "NP Fruška Gora". The subject of research is balance sheets and profit and loss accounts of company. In the conducted research, the elements of business indicators and business efficiency essential for the analysis of financial indicators of the PE "NP Fruška Gora" were analyzed. Data from the company's balance sheet and income statement were used as material. The following methods were used in the work: a. Financial indicators: liquidity, solvency, indebtedness and b. Performance indicators: productivity, efficiency, profitability. Based on the data from the balance sheet, we can see that the company used funds from credits or loans. By increasing the value of real estate, plant and equipment, and by reducing long-term receivables, the fixed assets of the company also increased, which as of 2018 increasing. The company operated with a business profit for 4 years in a row. In 2014, 2015, 2019, 2021, the company suffered a loss from financing. At no time was the company liquid due to high liabilities. In the period 2014-2021, the company was always solvent. The company needs to improve the labour productivity parameter, which is constantly decreasing. The company generally operates efficient from the point of view of indicators of overall and regular operations, and by observing the indicators of the economy of financing. If all obligations were to be reduced, the indebtedness factor would also be reduced.

Keywords: financial indicators, PE “Fruška Gora Natonal Park”, business

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INTRODUCTION

Financial statements are the basic product of the accounting of a company whose aim is to collect, record, classify and present all business changes that occurred in a company during a reporting period. Financial reports are a value presentation of the impact of those changes on the financial structure, business success and changes in cash flows of a company. Therefore, they represent the basis and subject of financial analysis (Knežević, *et al.,* 2017). Financial analysis includes the examination of everything that in any way represents the financial situation of the company. This means that it always includes different financial reports, primarily the balance sheet and the income statement (Barjaktarović, *et al*., 2021).

Fruška gora was declared a national park in 1960 in order to ensure permanent protection, located in Serbia. The area of active protection includes 27,762 ha.

The aim of the research is the financial analysis of the operations of the PE "NP Fruška Gora". The purpose of the research is to identify problems in business and indicate opportunities for improving the economic efficiency of the business of PE "NP Fruška Gora". The subject of research is balance sheets and profit and loss accounts of company.

MATERIAL AND METHODS

In the conducted research, the elements of business indicators and business efficiency essential for the analysis of financial indicators of the PE "NP Fruška Gora" were analyzed. Data from the company's balance sheet and income statement were used as material.

The following methods were used in the work:

–Financial indicators: liquidity, solvency, indebtedness.

–Performance indicators: productivity, efficiency, profitability.

Liquidity means the company's ability to meet its obligations to creditors on time (Ivaniš and Nešić, 2011). Solvency is defined as the ability of a company to meet its obligations, at any time, even from the bankruptcy estate. The total business assets of the company can be financed from different sources of financing, which are divided into borrowed and own according to the criteria of belonging (Ivaniš and Nešić, 2011). Productivity is an economic principle that expresses the aspiration or requirement to achieve a certain volume of production, volume of turnover, or volume of performed services with as little labor consumption as possible (Barać and Stakić, 2007, Keca, 2015). Efficiency, as one of the economic criteria for the success of production, expresses the desire to perform specific, quantitatively expressed, tasks of production and/or services with the lowest possible consumption of materials, means of work and labor (Barać and Stakić, 2007). Profitability is expressed as a requirement to achieve maximum income with as few resources as possible in the reproduction process.

In economic literature, it is usual to separate the balance sheet from the income statement, where the balance sheet is defined as an instrument for

showing the financial condition of the company understood in the sense of quantitative and qualitative changes in the disposal of funds. On the other hand, the income statement is defined as an instrument that aims to express the degree of business success in a given period of time (Knežević, *et al*., 2017).

Based on the data from Figure 1, it can be seen that the total assets since 2014. until 2017 slightly decreased, and that from 2018 until 2021 started to grow again. Real estate, plants, equipment and biological assets have the largest share in total assets.

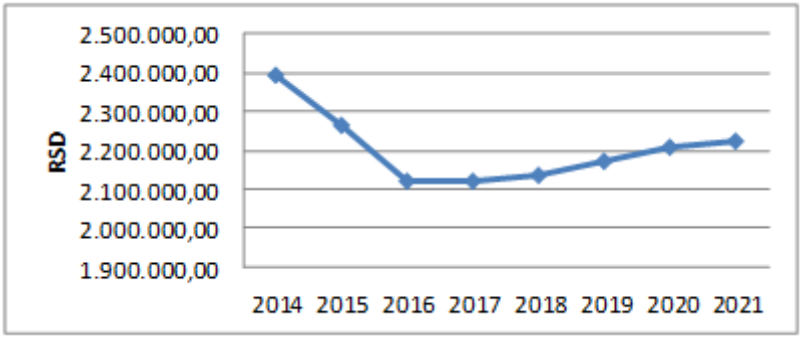

Figure 1. Balance sheet of PE "NP Fruška Gora" 2014/15.../16/17/18/19/20/21. (in RSD) - ASSETS - Fixed assets

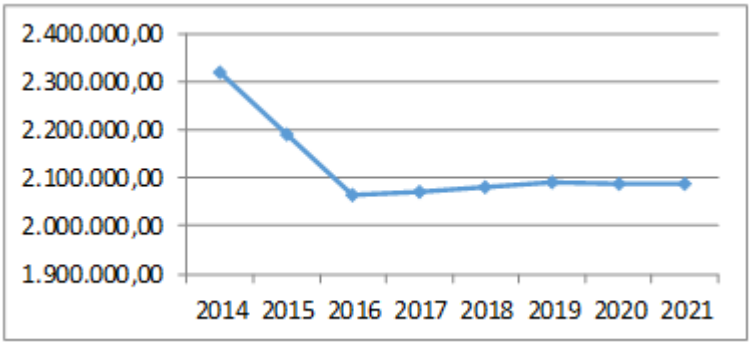

Figure 2. Balance sheet of PE "NP Fruška Gora" 2014/15.../16/17/18/19/20/21. (in RSD) - LIABILITIES – Capital

Figure 2 presents liabilities for the period from 2014 until 2021, where observe that the fixed capital in 2015-16 recorded a decrease, and since 2016 until 2021 constantly the same. It means that the value of state capital and fixed capital has not changed over the years. Reserves have been constantly increasing since 2017. Long-term provisions and liabilities were reduced in 2015 and 2016 and from 2017 until 2019 we have an increase, and then a decrease again in 2020 and in 2021.

This means that the company in the period 2017-2019 used other funds in the form of credits, loans and other long-term funds. Short-term liabilities have a dynamic movement from year to year, the highest value was recorded in 2018 and the lowest in 2021. This tells us that payables and trade payables have a variable trend in value.

Table 1 shows business income and business expenses. As we can see, business income was not always higher than business expenses, so the company operated with a loss in 2014, 2019, 2020, 2021, while in the period 2015-2018 recorded business income that was constantly growing.

Table 1. Balance sheet of PE "NP Fruška Gora" 2014/15.../16/17/18/19/20/21. (in RSD) - business income/expenses

	Position	2014	2015	2016	2017	2018	2019	2020	2021
1	Bussines income	368.392	355.068	391.196	379.893	371.375	351.652	328.882	345.949
2	Bussines expences	372.451	342.923	373.124	359.755	338.514	352.112	340.968	353.330
3	Bussines profit	/	12.145	18.072	20.138	32.861	/	/	/
4	Bussines loss	4059	/	/	/	/	460	12.086	7.381

Table 2 presents the financial income and financial expenses of PE "NP Fruška Gora". The company is in 2014, 2015, 2019 and 2021 recorded higher financial expenses than financial income and operated with a loss from financing, and in 2016, 2017, 2018 and 2020 recorded higher financial income than financial expenses and operated with a profit from financing, which in 2018 amounted to 3,583 dinars.

Table 2. Income statement of PE "NP Fruška Gora"2014/15.../16/17/18/19/20/21. (in RSD) – financial income/expenses

	Position	2014	2015	2016	2017	2018	2019	2020	2021
1	Bussines income	230	1.060	2.405	1.167	3.712	1.642	1.780	770
2	Bussines expences	2.423	1.411	1.026	1.054	129	4.275	622	1.419
3	Bussines profit	/	/	1.379	113	3.583	/	1.158	/
4	Bussines loss	2.193	351	/	/	/	2.633	/	649

Other revenues arise from the sale of securities, fixed assets, materials, surpluses, reduction of obligations to clients and state authorities. Other expenses that arise as a result of unforeseen events that the company cannot influence (deficits, funds, fines, etc.). The company made a profit in 2014, 2015, 2017, 2019, 2020 and 2021. The biggest profit was in 2020 from 34,573 Serbian dinars (RSD). As for losses, there were losses in 2016 from 13,590 RSD and in 2018 from 13,051 RSD.

RESULTS AND DISCUSSION

It can be seen that the company was not liquid at any moment of the observed period. The reason for all this is that short-term liabilities have a greater amount of value than all other balance sheet indicators (Figure 3).

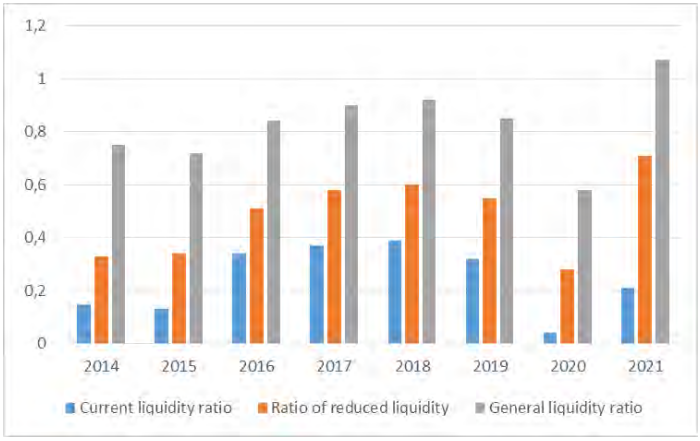

Figure 3. Movement of company liquidity

In figure 3, we can see that all three liquidities have the lowest coefficient in 2020. The reason for this is the decrease in cash, current assets and inventories in relation to short-term liabilities, which in 2020 had the second highest value in the observed period.

Figure 4 shows that the company is permanently solvent in the observed period, that is, the company is capable of paying all its obligations when they come due.

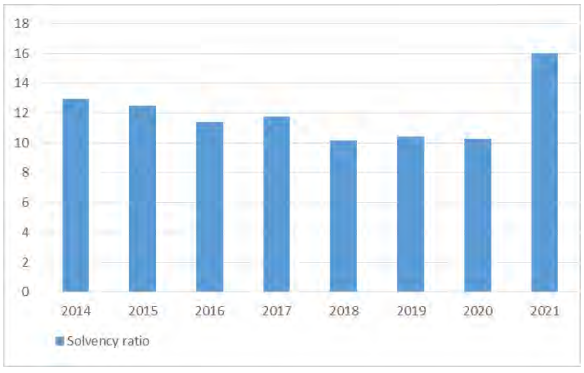

Figure 4. Trends in the company's solvency

On figure 4, we can see that the coefficients have a decreasing tendency from the beginning of the analysis until 2020. The reason for this is the increase in liabilities. By reducing liabilities in 2021, a dynamic increase in the Company's solvency ratio can be observed.

The analysis of the indebtedness of the PE "NP Fruška Gora" is presented through several financial elements (financial leverage, coefficient of own financing, indebtedness factor, independent financing indicator).
In figure 5, the financial leverage has oscillations in its movement, it only remained in the period 2018-2020 and recorded the highest value of 0.11, resulting in increased debts. The lowest value of this indicator was recorded in 2021 of 0.07 due to reduction in liabilities.

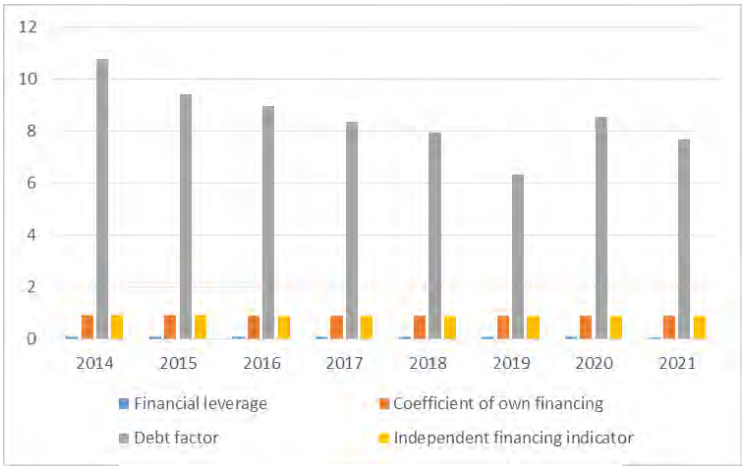

Figure 5. Trends in corporate indebtedness

Figure 5 shows that the indebtedness factor was at its peak at the beginning of the observed period, i.e. in 2014. after which its value began to decline until 2019. Then in 2020. an increase was recorded again in order to decrease again in 2021. The reason for such a movement is the change in the ratio of liabilities and total depreciation.

The company achieved the highest productivity in 2016 due to increased sales revenue. In the following years, we see that the company's productivity is falling, the reason being the decrease in sales revenue. The lowest value was recorded in 2021, regardless of the fact that the number of workers was reduced, because the income from sales did not stop falling.

Table 3. Analysis of company productivity

Position	2014	2015	2016	2017	2018	2019	2020	2021
Sales revenue	294.689	274.054	311.112	290.111	259.902	216.445	187.407	177.371
No. of workers	154	154	153	151	151	151	151	143
Productivity	1.913	1.779	2.033	1.921	1.721	1.433	1.241	1.240

As for the intensity of the productivity movement, graph 9 shows that the highest productivity was achieved in 2016. After that, a decreasing tendency can be observed until the end of the observed period, that is, until 2021 (Table 3).

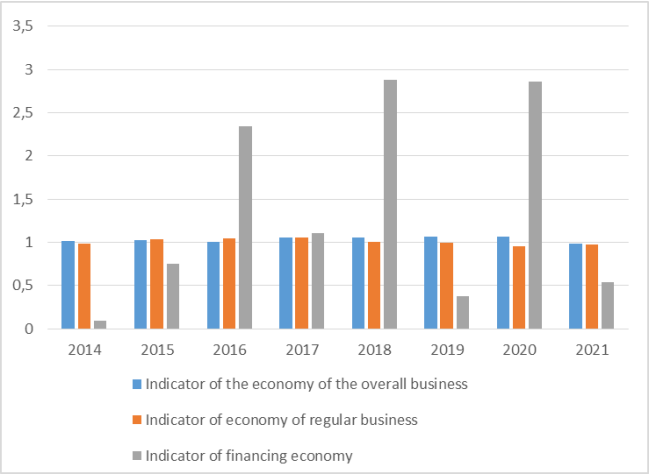

Figure 6. Trend of the company's efficiency

The analysis of the efficiency of the Company is expressed through several indicators (the efficiency indicator of the overall operation, the efficiency indicator of regular operations and the indicator of the efficiency of financing).

The overall business efficiency indicator tells us that the Company operates economically in all years except 2021, where total expenses are slightly higher than total revenues. The economic efficiency indicator of regular operations shows us that in 2014, 2020 and 2021, the company had higher business expenses compared to business income (Figure 6). On Figure 6, it can be seen the sharp growth of the financing economy indicator in 2016, 2018, 2020. The reason for the growth is the increase in financial income.

The company's goal is to maximize profit in the long term, and the goal of monitoring profitability indicators is to assess the degree of achievement of set goals (Spasić and Čerović, 2014).

Table 4. Analysis of the company's profitability

Position	2014	2015	2016	2017	2018	2019	2020	2021
Average net profit	1.279	2.391	5.181	8.085	12.198	17.070	13.879	5.466
Average equity	2.319.829	2.255.292	2.128.023	2.068.286	2.076.290	2.086.088	2.090.057	2.088.098
Return on equity	0,05	0,11	0,24	0,39	0,59	0,82	0,66	0,26

The profitable value of the Company increased from year to year until 2019 where it recorded the highest growth, and from 2020 it starts to decline, the

reason for all this is the change in own capital and net profit. There is a tendency to increase profitability until 2019. The drastic drop in profitability in 2021 is the result of a reduced net profit (Table 4).

CONCLUSIONS

Based on the financial analysis of PE "NP Fruška Gora", we came to the following conclusions:

- Based on the data from the balance sheet, we can see that the company used funds from credits or loans.
- By increasing the value of real estate, plant and equipment, and by reducing long-term receivables, the fixed assets of the company also increased, which as of 2018 increasing.
- The company operated with a business profit for 4 years in a row.
- In 2014, 2015, 2019, 2021, the company suffered a loss from financing.
- At no time was the company liquid due to high liabilities.
- In the period 2014-2021, the company was always solvent.
- The company needs to improve the labour productivity parameter, which is constantly decreasing.
- The company generally operates economically from the point of view of indicators of overall and regular operations, and by observing the indicators of the economy of financing, we see that the company in 2016, 2017, 2018, 2020 had a higher reference value than allowed and was not economical from this aspect.
- The highest value of profitability was in 2019.
- If all obligations were to be reduced, the indebtedness factor would also be reduced.

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INTERDEPENDENCE RELATIONSHIPS BETWEEN THE PRODUCTIVITY ELEMENTS IN WHEAT EAR AND THE PHOTOSYNTHETIC PIGMENTS IN LEAVES IN RELATION TO THEIR POSITION ON THE STEM

SUMMARY

The study analyzed the interdependence relationships between the productivity elements of the wheat ear and the content of photosynthetic pigments in the leaves, and described the causal relationships based on equations and graphic models. The study took place in the Didactic and Experimental Resort of the Life Science University "King Michael I" from Timisoara, Romania. The biological material was represented by the Solehio wheat variety. The content of chlorophyll (Chl) and carotenoids (Car) was determined in the booting stage (45 – 47 BBCH code) in the five leaves on the stem (L1 to L5), according to the structural units (phytomers) specific to the Solehio variety. At physiological maturity (99 BBCH code), ear samples were taken and productivity elements were determined: spikelets number in ear, SN-E; fertile spikelets number in ear, FSN-E; grain number in spikelet, GN-S; grain number in ear, GN-E; grain weight in ear, GW-E; mean weight of a grain, MWG. Very strong and strong correlations were recorded between Chl and Car at the level of the studied leaves (e.g. $r=0.937$ in the case of L3, $p<0.001$; $r=0.859$ in the case of L5, $p<0.01$) and between the elements of productivity and leaves (e.g. between GN-E and Chl L5, $r=0.886$, $p<0.001$; between GN-S and Chl L5, $r=0.857$, $p<0.01$; between GW-E and Chl L5, $r=0.825$, $p<0.01$). The regression analysis facilitated obtaining an equation and 3D models and in the form of isoquants, which described the variation of the productivity elements in relation to Chl and Car at the level of the flag leaf (L5), under statistical safety conditions (R^2, p and RMSEP).

Keywords: correlations, chlorophyll, carotenoids, phytomers, wheat, ear, productivity elements

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INTRODUCTION

Wheat is a plant of major importance within crop plants, for reasons of food resources, fodder, for industrialization, bakery, pastry, etc., and it is a crop plant with a long history in relation to humanity (Grote *et al.,* 2021; Erenstein *et al*., 2022).

As a result of its importance, wheat has been widely studied from the perspective of genetic aspects and breeding programs (Gao *et al.,* 2021; Govta *et al.,* 2022), physiological aspects (Mohan and Gupta, 2015), soil fertilization and plant nutrition aspects (Hirzel *et al.,* 2022; Jat *et al*., 2022), productivity elements (Datcu *et al.,* 2019; Jat *et al*., 2022), production quality, but also in relation to their interactions (Govta *et al.,* 2022; Jat *et al.,* 2022).

Interaction "genotype x environment" or complex interactions of the type "genotype x environment x technological inputs" were studied and quantified in different experimental conditions regarding wheat culture (Tofighi *et al*., 2021; Todorova *et al*., 2022).

The leaves of wheat plants have a linear shape, with variations from juvenile to adult plant (Dornbusch *et al*., 2011) variable number in relation to the genotype and vegetation conditions, with a basal arrangement (the basal leaves, in the first stages of vegetation) but also an alternate arrangement, on the height of the stem, related to the repetitive structural units (phytomers) of the stem (Kirby, 2002).

The appearance, arrangement and formation / extension of wheat leaves has been studied in relation to genetic factors (Yang *et al.,* 2021; Mehla *et al*., 2022), but also to environmental and technological factors, such as fertilizers (Hay and Wilson, 1982; Kubar *et al.,* 2022; Merrium *et al*., 2022).

The content of photosynthetic pigments is important in relation to the photosynthesis process, in order to convert solar energy into biochemical energy and specific metabolic compounds (Lu *et al.,* 2001; Liu *et al*., 2020).

The content of photosynthetic pigments in wheat varies in relation to the genotype (Javed *et al.,* 2022), environmental factors and vegetation conditions (Xiong *et al.,* 2015), the position of the leaves on the plant (may much studied flag leaf) (Roy *et al.,* 2021; Yang *et al.,* 2022), with fertilization and plant nutrition status (Zhang *et al*., 2021), water regime (Yang *et al.,* 2022), the state of plant health (Cai *et al.,* 2021), vegetation stages of plants (Zhang *et al*., 2009; Li *et al*., 2022), stress factors (Roy *et al*., 2021), but also interactions of factors, such as "genotype x environment" or "genotype x environment x technological inputs".

At the same time, it was found in some studies the variation of the spatial distribution of the photosynthetic pigments (eg chlorophyll) in the leaves, in relation to the geometry of the leaf (especially the length) but also with various other factors (Borsuk and Brodersen, 2019; Huang *et al*., 2023).

Wheat is cultivated with a predilection for the production of grains, so that the productivity elements of the wheat ear present the main category of parameters at the level of the plant and the crops and have been studied in relation to the genotype (Wolde *et al.,* 2019), and vary in relationship with the complex

interaction "genotype x environmental factors x culture technology" (Marcos-Barbero *et al.,* 2021; Nazarenko *et al*., 2022).

The present study analyzed the content of photosynthetic pigments in the leaves of wheat, the Solehio variety, in relation to the position of the leaves on the plant stem, productivity elements in the ear, and interdependence relationships between the productivity elements in relation to the content of photosynthetic pigments in the leaves.

MATERIAL AND METHODS

The study evaluated the productivity elements in the wheat ear in relation to the content of photosynthetic pigments in the leaves on the stem of the wheat plants.

The study took place in the Didactic and Experimental Resort of the Life Science University "King Michael I" from Timisoara, Romania. The biological material was represented by the Solehio wheat variety, cultivated on a medium-fertility chernozem type soil, non-irrigated system, agricultural year 2021 – 2022, Figure 1 (ESRI, 2014).

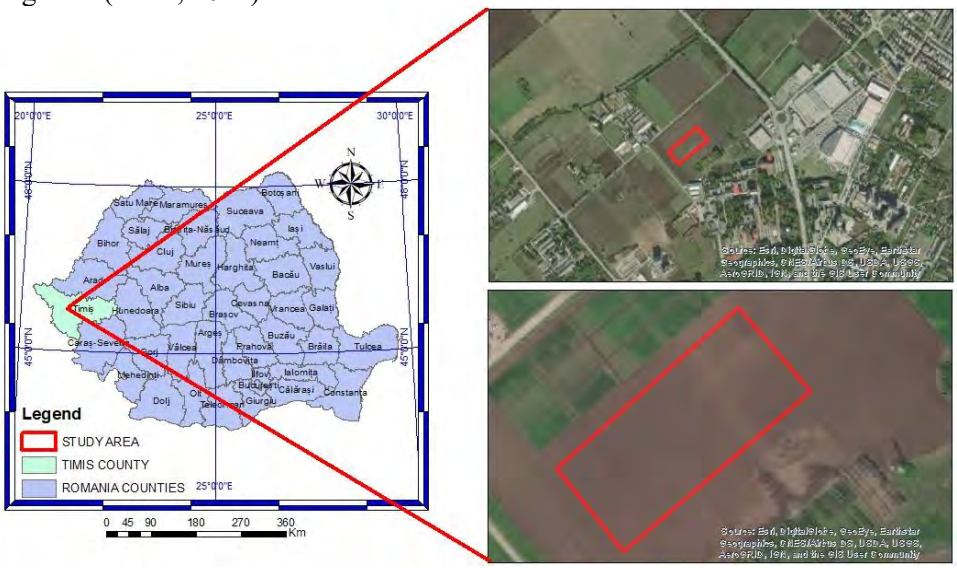


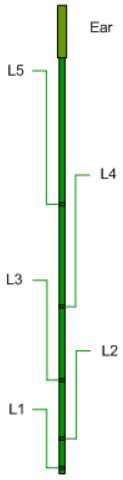
Figure 1. The location of the study

For the study, 10 wheat plants each (the main plant) were considered, randomly, from ten control points. The leaves related to the nodes arranged on the height of the stem of the wheat plants were analyzed, within the repetitive units (phytometers) of the formation of the stem of the wheat plant (Kirby, 2002; Gebbing, 2003). Between 6 and 16 such units, "phytomers or repeating units" (a node, a leaf, an elongated internode) were reported (Kirby, 2002).

In the case of the Solehio variety, 5 such units were identified, and the leaves were numbered starting from the base of the plants, in the order of the nodes and internodes (the units from the base to the top of the plant) - leaf 1 (L1),

leaf 2 (L2), leaf 3 (L3), leaf 4 (L4), and Leaf 5 - flag leaf (L5), figure 2a. Determination of photosynthetic pigments was done at Principal growth stage 4: Booting (45 – 47 BBCH code) (Meier, 2001). The content of photosynthetic pigments was determined by non-destructive methods; chlorophyll (Chl) with a SPAD 502Plus portable chlorophyll meter (KONICA MINOLTA), and for the carotenoid content (Car) the ACM-200 plus device (OPTI-SCIENCES) was used.

At physiological maturity, Senescence (99 BBCH code), spike samples were collected from the studied plants (E1 to E10), the main plant, and the productivity elements were determined: spikelets number in ear, SN-E; fertile spikelets number in ear, FSN-E; grain number in spikelet, GN-S; grain number in ear, GN-E; grain weight in ear, GW-E; mean weight of a grain, MWG, figures 2(b), (c).



Elements studied in the wheat ear

spikelets number in the ear (SN-E)
number of fertile spikelets in the ear (FSN-E)
grain number in spikelet (GN-S)
grains number in the ear (GN-E)
grains weight in the ear (GW-E)
mean weight of a grain (MWG)

(a) (b) (c)

Figure 2. Plant and ear of wheat, Solehio variety; (a) layout scheme of analyzed leaves; (b) spike of the main plant; (d) productivity elements analyzed at the ear level

The interdependence relationships between the content of photosynthetic pigments (Chl, Car) at the level of leaves L1 – L5, and the productivity elements studied at the ear level (SN-E, FSN-E, GN-S, GN-E, GW-E, MWG) were evaluated.

Descriptive statistical analysis, correlation analysis, regression analysis were used, and for the significance of the results, the correlation coefficient (r), the regression coefficient (R^2), RMSEP, equation (1), and the statistical safety parameter (p , *p<0.05; **p<0.01; ***p<0.001).

$$\mathrm{RMSEP} = \sqrt{\frac{1}{n}\sum_{j=1}^{n}\left(y_j - \hat{y}_j\right)^2} \qquad (1)$$

The statistical processing and analysis of the experimental data was done with the calculation module in EXCEL, and with the softwares PAST (Hammer *et al.,* 2001), Wolfram Alpha (2020), and JASP (2022).

RESULTS AND DISCUSSION

The photosynthetic pigments, chlorophyll (Chl) and carotenoids (Car) were determined at bootting stage (45 – 47 BBCH code) on each leaf (L1 to L5), on the five units of the stem, specific to the Solehio wheat variety. In the case of the L1 leaf, the chlorophyll content varied between 9.60-12.70±0.32 SPAD units, and the carotenoid content varied between 3.60-4.10±0.06 units. In the case of the L2 leaf, Chl varied between 30.80-49.65±1.91 SPAD units, and Car varied between 5.30-8.70±0.38 units. In the case of the L3 leaf, Chl varied between 43.70-60.80±1.58 SPAD units, and Car varied between 8.70-13.70±0.52 units. In the case of the L4 leaf, Chl varied between 49.90-58.80±1.05 SPAD units, and Car varied between 9.50-15.50±0.64 units. In the case of the L5 leaf, Chl varied between 46.70-55.20±0.86 SPAD units, and Car varied between 9.70-15.40±0.62 units. The complete series of values recorded at the foliar level, on the five floors of the stem of the wheat plants, at split (booting, 45 – 47 BBCH code) are presented in table 1.

Table 1. Photosynthetic pigments values in leaves of wheat plants, Solehio wheat variety

Trial	Main stem plants									
	Chl-L1	Car-L1	Chl-L2	Car-L2	Chl-L3	Car-L3	Chl-L4	Car-L4	Chl-L5	Car-L5
P1	10.10	3.75	41.60	6.85	48.90	10.60	51.20	11.60	47.70	9.70
P2	12.40	4.00	48.10	7.60	54.30	10.80	52.50	11.20	54.90	15.40
P3	11.90	4.10	45.10	7.70	57.60	13.70	51.90	13.20	55.20	15.20
P4	12.70	3.90	49.65	8.70	60.80	13.70	58.80	15.50	52.40	12.60
P5	10.80	4.10	38.50	5.30	52.50	11.70	49.90	10.80	51.90	11.10
P6	10.60	3.70	33.90	5.60	53.40	11.10	58.10	14.00	52.50	10.60
P7	11.00	3.60	43.80	6.40	47.00	9.10	56.80	15.20	52.80	12.50
P8	9.60	4.00	30.80	5.30	53.10	10.80	50.30	9.50	46.70	10.05
P9	11.10	3.70	46.10	7.90	43.70	8.70	55.70	14.60	51.60	11.90
P10	10.30	3.80	42.60	6.20	50.10	10.50	51.70	12.10	50.75	11.30
SE	±0.32	±0.05	±1.91	±0.37	±1.57	±0.52	±1.05	±0.64	±0.86	±0.62

Note: Chl – chlorophyll content and Car – carotenoid content; L1 to L5 - the position of the leaves on the plant; P1 to P10 – wheat plants samples studied; SE – Standard Error

At the same plants, the ears were harvested at the moment of physiological maturity, and productivity elements were determined. The number of spikelets in

the ear (SN-E) varied between 18.00-19.00±0.17. The number of fertile spikelets in the ear (FSN-E) varied between 16.00-17.00±0.16. The number of grains in the ear (GN-E) varied between 33.00-53.00±2.30. The average number of grains in the spikelet (GN-S) varied between 2.030-3.118±0.128. The average weight of the grains in the ear (GW-E) varied between 1.1360-2.1310±0.1109 g. The average weight of a grain in the ear (MGW) varied between 0.0034-0.0402±0.00078 g. The series of values recorded for the productivity elements in the ear, the Solehio wheat variety, are presented in table 2.

Table 2. Average values of the productivity elements in the ear, Solehio wheat variety

Trial		Main stem ear					
Plant	Spike	SN-E	FSN-E	GN-S	GN-E	GW-E	MWG
P1	E1	19	16	2.13	34	1.140	0.03353
P2	E2	18	17	3.06	52	1.878	0.03612
P3	E3	19	17	3.12	53	2.131	0.04021
P4	E4	18	16	2.81	45	1.502	0.03338
P5	E5	19	17	2.11	38	1.276	0.03753
P6	E6	19	16	2.69	43	1.687	0.03923
P7	E7	18	17	2.95	51	1.933	0.03790
P8	E8	18	16	2.03	33	1.136	0.03442
P9	E9	19	17	2.78	49	1.887	0.03851
P10	E10	18	17	2.65	44	1.713	0.03893
SE		±0.17	±0.16	±0.13	±2.30	±0.11	±0.00078

Note: SN-E – spikelets number in ear; FSN-E – fertile spikelets number in ear; GN-S – grain number in spikelet; GN-E – grain number in ear; GW-E – grain weight in ear; MWG – mean weight of a grain (g); SE – Standard Error; E1 to E10 – ears sample corresponding to plant sample P1 – P10

The graphic distribution of the data series recorded for the photosynthetic pigments in the analyzed L1-L5 leaves and the productivity elements at the level of the spike are shown graphically in figures 3 and 4, in boxplot format.

The content of photosynthetic pigments in the leaves of wheat, Solehio variety, at the time of determination (45 – 47 BBCH code) varied in relation to the position of the five leaves on the stem (L1 to L5), according to a model described by equation (2) in the case chlorophyll content (Chl, R2=0.976, p=0.0236), respectively equation (3) in the case of carotenoid content (Car, R^2=0.970, p=0.0296). The graphic distribution of Chl and Car values in relation to the position of the leaves L1 to L5 on the plant stem, and the graphic expressions of equations (2) and (3), are presented in figure 5.

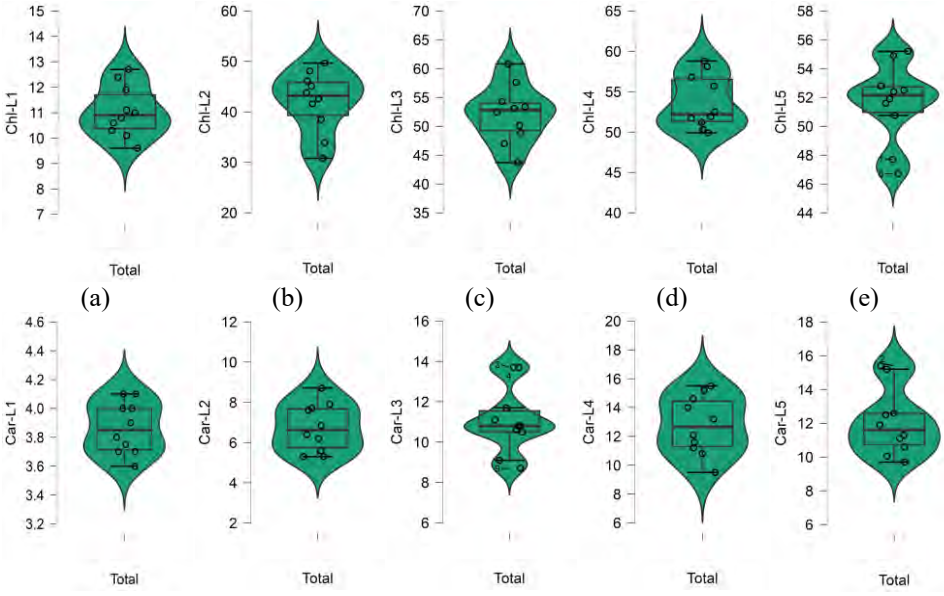


Figure 3. Distribution in boxplot format of photosynthetic pigments values in wheat leaves, Solehio variety; (a) Chl-L1; (b) Chl-L2; (c) Chl L-3; (d) Chl L-4; (e) Chl L-5; (f) Car L-1; (g) Car L-2; (h) Car L-3; (i) Car L-4; (j) Car L-5

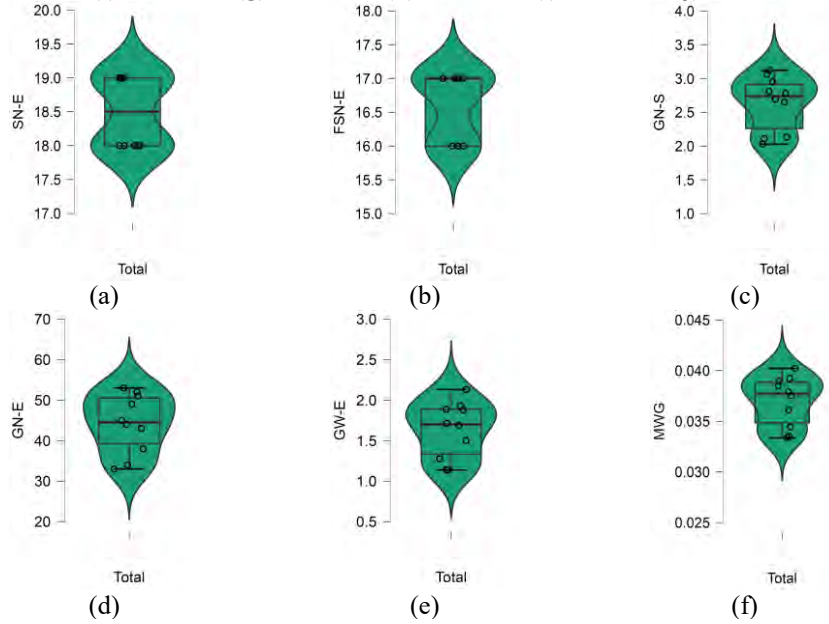


Figure 4. The distribution in boxplot format of the values of the productivity elements at ear level, Solehio wheat variety; (a) SN-E – spikelets number in ear; (b) FSN-E – fertile spikelets number in ear; (c) GN-S – grains number in spikelets; (d) GN-E – grains number in ear; (e) GW-E – grains weight in ear; MWG – mean weight of a grain

$$Chl = -5.328x^2 + 41.25x - 23.05 \quad (2)$$

$$Car = -0.7036x^2 + 6.456x - 2.328 \quad (3)$$

where: x – Leaf position

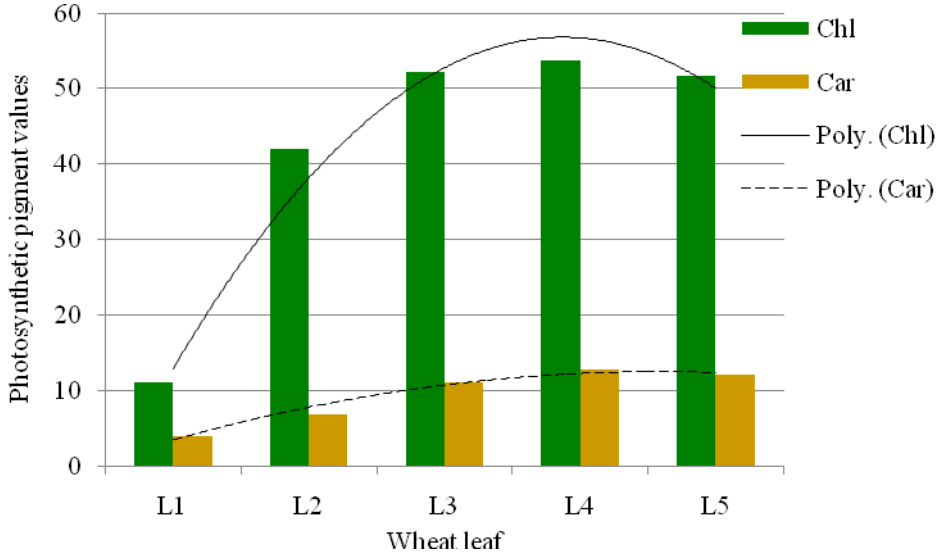


Figure 5. Distribution of photosynthetic pigments (Chl and Car) in relation to the position of the leaves on the stem, Solehio wheat variety (45 – 47 BBCH code)

The correlation analysis highlighted different levels of correlation, positive or negative, between the photosynthetic pigments at the level of the 5 studied leaves on the height of the plants, between the productivity elements at the ear, as well as between the productivity elements and the photosynthetic pigments, under statistical safety conditions (* $p<0.05$; ** $p<0.01$; *** $p<0.001$), figura 6.

At the level of photosynthetic pigments, very strong, positive correlations were recorded between Chl and Car ($r=0.937$) at the level of the L3 leaf ($p<0.001$). Strong correlations were identified between Chl and Car at the L2 leaf level ($r=0.887$), at the L4 leaf level ($r=0.893$) under conditions of $p<0.001$, respectively between Chl L2 and Chl L1 ($r=0.809$), between Chl L5 and Chl L1 ($r=0.801$), between Car L5 and Chl L1 ($r=0.824$) and between Chl L5 and Car L5 ($r=0.859$) under conditions of $p<0.01$.

At the level of productivity elements at the spike, very strong positive correlations were recorded between GN-E and GN-S ($r=0.970$), between GW-E and GN-E ($r=0.965$), between GE-E and GN-S ($r=0.938$) under conditions of $p<0.001$. Some moderate or weak correlations were also recorded, under statistical safety conditions ($p<0.05$).

Between productivity elements and photosynthetic pigments, certain correlations were also recorded, of different levels of intensity and statistical certainty.

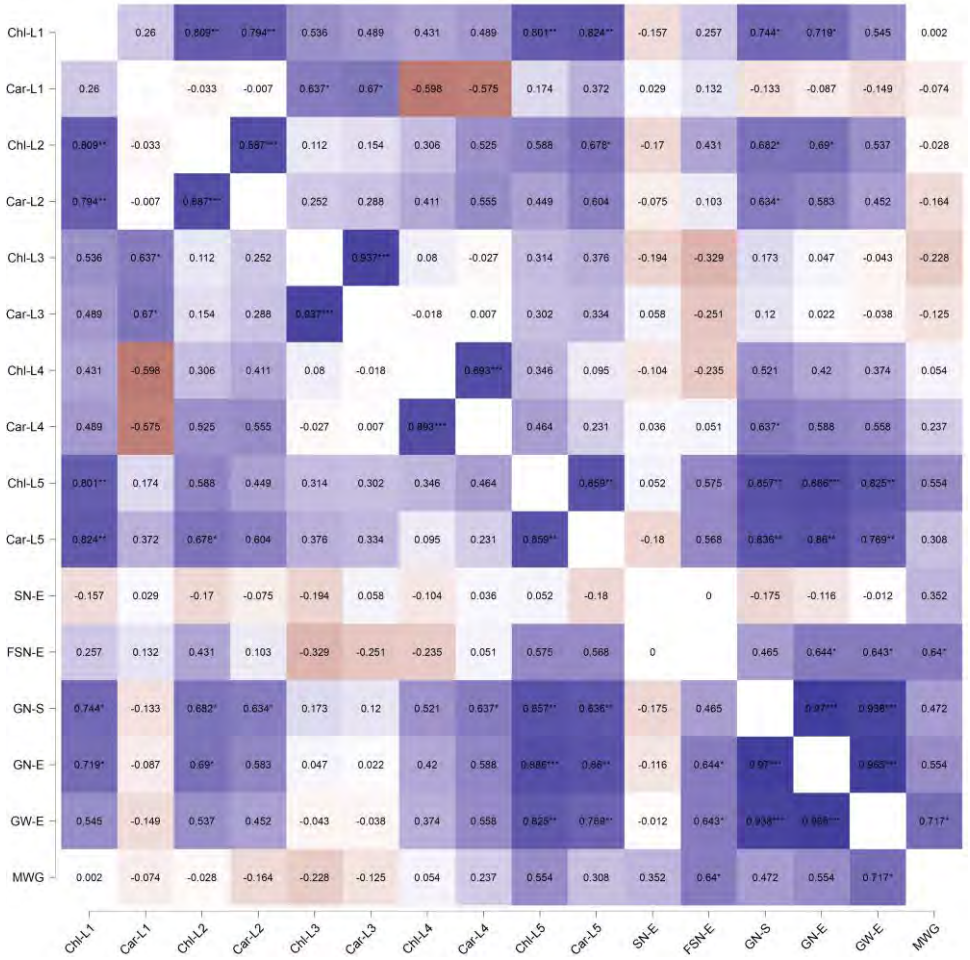


Figure 6. The level of correlations, in the form of Pearson's r heatmap, between photosynthetic pigments, leaves L1 to L5, and productivity elements in the ear, Solehio wheat variety

Strong, positive correlations were recorded between GN-E and Chl L5 ($r=0.886$, $p<0.001$), between GN-E and Car L5 ($r=0.860$, $p<0.01$), between GN-S and Chl L5 ($r=0.857$, $p<0.01$), between GN-S and Car L5 ($r=0.836$, $p<0.01$) and between GW-E and Chl L5 ($r=0.825$, $p<0.01$). Moderate, positive correlations were recorded between GW-E and Car L5 ($r=0.769$, $p<0.01$), between GN-S and Chl L1 ($r=0.744$, $p<0.05$) and between GN-E and Chl L1 ($r=0.719$, $p<0.05$). Weak correlations were recorded between GN-S and Chl L4 ($r=0.637$, $p<0.05$), between GN-S and Chl L2 ($r=0.682$, $p<0.05$), between GN-S and Car L2 ($r=0.634$, $p<0.05$) and between GN-E and Chl L2 ($r=0.690$, $p<0.05$). Also, other correlations, positive or negative, were recorded between the productivity elements at the ear and the photosynthetic pigments recorded at the level of the leaves on the stem of the wheat plants (L1 to L5).

Starting from these recorded correlation levels, regression analysis was used to evaluate and describe through equations and graphic models, the variation of productivity elements in relation to the photosynthetic pigments in the standard leaf. Thus, the variation of productivity elements in relation to the content of photosynthetic pigments in the standard leaf was described by equation (4), under statistical safety conditions. The values for the coefficients of equation (4) and the statistical safety parameters (R^2, p, RMSEP), in relation to each element of productivity, are presented in table 3. Based on the values of the coefficients of equation (4), 3D models were obtained and in the form of isoquants, which graphically represented the variation of productivity elements in the ear in relation to Chl and Car from the flag leaf. For example, the graphic models for the variation of FSN-E in relation to Chl and Car (L5), figure 7, the variation of GW-E in relation to Chl and Car (L5), figure 8, and the variation of MWG in relation to Chl and Car are presented (L5), figure 9.

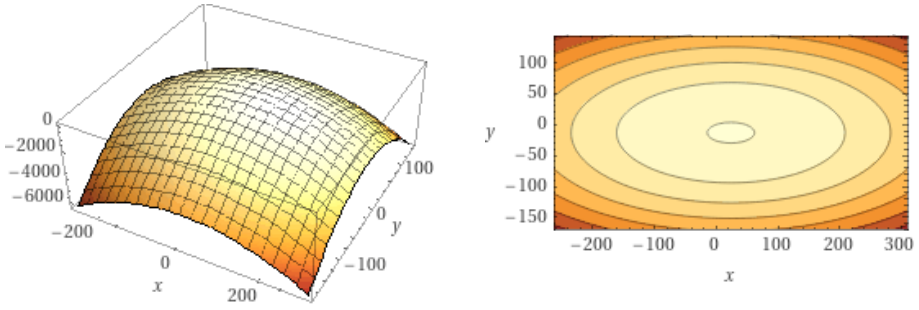
$$Y = ax^2 + by^2 + cx + dy + exy + f \quad (4)$$

where: Y – productivity element in ear (detailed in table 3);
x – Chl (x-axis); y – Car (y-axis); a, b, c, d, e, f – coefficients of the equation (4), table 3.

Table 3. The values of the coefficients of equation (4) and of the statistical safety parameters

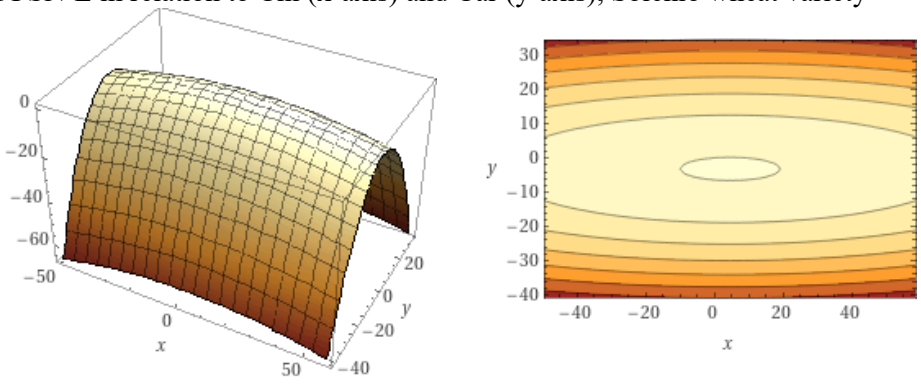
Y productivity elements in ear	Coefficients of equation (4)						Statistical parameters		
	a	B	c	d	e	f	R^2	p	RMSEP
SN-E	-0.07870	-0.20579	4.05314	-16.13653	0.39053	0	0.999	0.001	0.32677
FSN-E	-0.03055	-0.15877	1.45439	-4.15443	0.15462		0.999	0.001	0.36414
GN-S	0.00644	-0.00788	-0.34179	1.60984	-0.02423		0.995	0.001	0.17023
GN-E	0.00702	-0.83028	-2.39607	10.04188	0.24207		0.997	0.001	2.46553
GW-E	-0.00232	-0.04234	0.02091	-0.26682	0.02597		0.988	0.001	0.17568
MWG	-0.00005	-0.00023	0.00327	-0.01259	0.00032		0.997	0.001	0.00178

Within the productivity elements at the spike level, it was found that SN-E had very low correlations with photosynthetic pigments from L1 to L5 leaves. The number of spikelets in the ear is a genetically defined character (Li *et al*., 2021; Zhang *et al*., 2022), and in the study conditions, a very low interdependence of the SN-E parameter with the content of photosynthetic pigments in the arranged leaves was found on the plant stem (L1 to L5), considered in the study. In some studies, it was hypothesized that the number of differentiated spikelets correlates with the N nutrition of the plant in the late stage of differentiation in the late stage of spikelet differentiation (Wada, 1969), but also in different stages of the reproductive period (Kamiji *et al*., 2011), and the number of degenerate spikelets correlates with the evolution of the crop from then until heading.



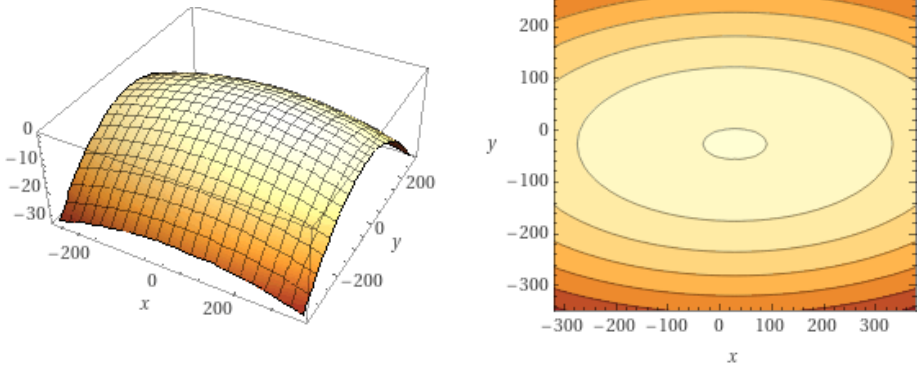
(a) (b)

Figure 7. 3D models (a) and in the form of isoquants (b) regarding the variation of FSN-E in relation to Chl (x-axis) and Car (y-axis), Solehio wheat variety



(a) (b)

Figure 8. 3D models (a) and in the form of isoquants (b) regarding the variation of GW-E in relation to Chl (x-axis) and Car (y-axis), Solehio wheat variety



(a) (b)

Figure 9. 3D models (a) and in the form of isoquants (b) regarding the variation of MWG in relation to Chl (x-axis) and Car (y-axis), Solehio wheat variety

In the case of the FSN-E parameter, it showed positive correlations with the chlorophyll content in the L2 leaf (r=0.431), negative correlations with the photosynthetic pigments in the L3 leaves (r=-0.329 in the case of Chl-L3, r=-

0.251 in the case Car-L3) and L4 leaves (r=-0.235 in the case of Chl-L4). Even if they are correlations of weak intensity, they show the interdependent relationship between the fertile spikelets and the photosynthetic pigments in the leaves on the stem position, respectively with the vegetation state of the plants in different stages of plant growth.

The number of grains in the spikelet (GN-S), the number of grains in the ear (GN-E), the weight of the grains in the ear (GW-E) are determined genetically (Christiansen et al., 2011; Nadolska-Orczyk *et al*., 2017), but also varies in relation to environmental factors or elements of technology, fertilization, irrigation, etc. (Zhou *et al*., 2018; Ma *et al*., 2022).

The productivity elements GN-S, GN-E and GW-E presented strong correlations with the photosynthetic pigments in the L5 (flag) leaf, a fact that confirms the importance of the flag leaf in defining the productivity elements in the ear and the quality of wheat production. The obtained results are in agreement with other studies, which presented the importance and contribution of photosynthetic pigments to the formation and filling of grains in the wheat ear (Sanchez-Bragado, 2016; Wang *et al*., 2016).

Significant positive correlations, between the number of grains in the spikelet and the number of grains in the ear (r=0.607), and the weight of the grains in the spikelet (r=0.573) were also reported in other comparative analysis studies of some wheat genotypes (Xhulaj Bode and Koto, 2022), in studies on the main components in the formation of wheat production (Nazarenko *et al*., 2021), or in studies on the relationship with fertilization (Rawashdeh and Sala, 2016; Ma *et al.,* 2022).

CONCLUSIONS

The content of photosynthetic pigments (Chl, Car) determined of the Solehio wheat variety, in the booting stage (45 – 47 BBCH code), recorded differentiated values, gradually increasing, from the basal leaf (L1) to the standard leaf (L5).

For the productivity elements at the ear level, correlations of different intensity levels (strong, moderate) were obtained with the photosynthetic pigments recorded in the leaves, in relation to the position of the leaf on the stem (structural units, L1 to L5), under conditions of statistical certainty ($p<0.001$, $p<0.01$).

Through the regression analysis, models were obtained in the form of an equation and graphic models (3D and in the form of isoquants) that described the variation of the productivity elements in relation to the photosynthetic pigments (Chl and Car) at the level of the standard leaf (L5), in conditions of statistical safety (R^2, p and RMSEP).

The obtained data show the differentiated contribution of the photosynthetic pigments in the leaves (L1 to L5, in relation to the structural units of the stem) at the determined moment (booting stage, 45-47 BBCH code), to the formation of the productivity elements in the ear.

The approach model can be adapted and extended to other straw cereals, at different stages of vegetation, for comparative studies and analysis.

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IMPACT OF SOIL MANAGEMENT PRACTICE ON THE ABUNDANCE OF MICROBIAL POPULATIONS

SUMMARY

Microorganisms in the soil have a very important role because they participate in numerous processes. Intensive and/or inadequate use of the soil leads to disturbance of the plant - microbial interactions, a decline in productivity, and degradation. The abundance and microbiological activity of a certain ecosystem are considered indicators of soil fertility. In this paper, surface (0-20 cm) and subsurface (20-40 cm) samples of grassland, agricultural soil, forest soil and coal-mine-affected soil at the Banovići municipality (Tuzla Canton, Bosnia and Herzegovina) taken in October 2021 and April 2022 were used for chemical and microbiological characterization. Chemical analyses were performed using the standard methodology, while the microbial count was determined using the agar plate method. Enzyme production was expressed through dehydrogenase activity. The lowest pH value was recorded in forest soil, while the highest in the grassland. In all samples, microbial abundance decreased with increasing soil depth. The lowest microbial activity was observed in coal mine-affected soil. The highest value of the total number of bacteria and ammonifiers was recorded in forest soil. Oligonitrophiles were most abundant in agricultural soil, while the number of actinomycetes was highest in grassland. Dehydrogenase activity was highest in forest and agricultural soil. In most of samples, microbial abundance was higher in spring, while dehydrogenase activity was higher in autumn. This research confirms the impact of land use on microbial abundance as parameter of soil quality.

Keywords: soil, chemical characterization, microbial abundance, soil dehydrogenase activity

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INTRODUCTION

Soil is a three-phase system that provides the location for the life of many organisms (Nielsen *et al.* 2015) and supports their development through an accumulation of essential nutrients (Brevik and Sauer 2015). For more than 12000 years, the human population has managed soils through agricultural practice (Zeder 2011), which, due to the dramatic increase of Earth's population, became intensive over several last decades. This intensification led to an increase in fertilizer application in plant production; however, soil amendments rarely may replace the nutrient depletion during plant development, which contributes to agroecosystem degradation (Tan *et al*. 2005; Vitousek *et al*. 2009).

Vitousek *et al.* (2010) and Walker and Syers (1976) found low phosphorus availability in many soils, while Du *et al.* (2020) reported limitation in nitrogen content, which suggests the shifts in soil quality recommended for plant production (Silver *et al.* 2021). Thus, the reduction of land degradation and restoration of degraded soils are recommended to maintain the agroecosystem, increase plant productivity and provide food production (Bouma and Montanarella 2016; Keesstra *et al.* 2016). These postulates are integrated in the „2030 Agenda for Sustainable Development", which defines goals associated with soil management and long-term soil restoration (Keesstra *et al.* 2016).

According to Muñoz-Royas (2018), soil quality indicators can be divided into three groups: physical, chemical, and biological. The appropriate selection and application of soil indicators are very important for the realization of Sustainable Development goals and defining of ecosystem services (Costantini *et al.* 2016), such as nutrient cycling (Anaya-Romero *et al*. 2016; Pereira *et al.* 2018). Bender *et al.* (2016) and Wagg *et al*. (2014) described the role of microorganisms in nutrient cycling, ecosystem functions, and organic matter decomposition. Nannipieri *et al*. (2003) suggest that microbiological and enzymatic indicators are often used in the estimation of soil quality.

Although the microbial populations in soil are abundant and diverse, research studies are often focused on the impact of land use on bacterial and fungal count (Banerjee *et al.* 2019; Estendorfer *et al*. 2017; Tardy *et al*. 2015). An abundance of microbial populations has been frequently studied in grassland and forest soils (Lauber *et al*. 2013; Rasche *et al*. 2011; Stres *et al.* 2008), as well as in coal mine fields (Zhang *et al*. 2022). In addition, agricultural practices; fertilization, pesticide application, and irrigation affect the microbial diversity of soil and ecosystem functions (Ding *et al*. 2013). Furthermore, land use is one of the crucial parameters of the physical, chemical, and biological characteristics of soil (Fedele *et al*. 2018). Thus, the estimation of microbial indicators should be associated with the determination of the chemical properties of soil (He *et al.* 2021).

The objective of this paper was to determine the impact of land use shifts on the microbial count and some chemical parameters of soil.

MATERIAL AND METHODS

Samples of grassland (G), agricultural soil (A), forest soil (F), and coal-mine-affected soil (C) at the Banovići municipality (Tuzla Canton, Bosnia, and Herzegovina) were used for chemical and microbiological characterization. Soil sampling (0-20 and 20-40 cm) was performed in October 2021 and April 2022. Chemical analyses were performed at the start of experiment using the following methodology:

i. pH value in water and 1M KCl was measured according to ISO 10390:2005 standard;
ii. The humus content was determined using Mineev *et al.* (2001) method and;
iii. Available P and K content by Egner et al. (1960) method.

A microbial abundance of soil samples was determined using the agar plate method. The total number of bacteria (TNB) was determined using 0.1xTSA (tryptic soy agar), the number of ammonifiers (AM) was determined on nutrient agar (Torlak, Serbia), oligonitrophiles (ON) on Fyodorov`s agar, and actinomycetes (ACT) on starch ammonia agar. Incubation was carried out in an incubator (Binder, Germany). The incubation period for bacteria was five days at 30°C, while for actinomycetes 12 days at 30°C. The microbial number was expressed as CFU (colony forming units) per gram of dry soil (after drying in the oven at 105°C for 2 hours).

Estimation of dehydrogenase activity (DHA) was performed using Kasida *et al*. (1964) method and expressed as μg of triphenylformazan (TPF)/g/h.

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained values T-test ($p<0.05$) was performed.

RESULTS AND DISCUSSION

The results of this study showed variations in the chemical properties of samples, depending on management practices and soil depth (Table 1). In FS, the lowest pH value and available P content compared with other samples were noticed. On the other hand, the highest humus content was detected in the same sample. According to Wilpert (2022), forest soil has higher humus content than arable soils. Rozek *et al*. (2020) suggest that forest plots were characterized by lower pH values compared with other plots, probably due to organic matter degradation and the presence of root exudates (Schawe *et al*. 2007).

The highest pH value was detected in G. Schnoor *et al*. (2015) also found a high pH value in grassland, which is in agreement with our results. Organic matter content is lower in coal mine-affected soils compared with unmined soils (Guo *et al.* 2018); on the other hand, the same authors found a low potassium content in mined soils, which is opposite to our findings. However, Essandoh *et al.* (2021) found higher levels of K, Mg, and Na in mined sites.

Table 1. Chemical characterization of soil samples

sample	depth (cm)	pH H_2O	pH 1M KCl	humus (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
G	0-20	8.10	7.00	5.22	12.9	4.0
	20-40	7.60	6.90	3.75	7.5	2.3
A	0-20	7.95	6.01	3.85	27.4	12.0
	20-40	7.80	5.87	2.96	10.1	7.0
F	0-20	5.15	3.75	9.50	9.8	13.0
	20-40	4.90	3.59	5.70	3.2	6.0
C	0-20	7.96	6.20	1.66	22.6	19.0
	20-40	7.14	6.07	0.92	10.4	10.0

Legend: G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil

Table 2. Bacterial abundance (x 105 CFU/g) in soil samples

S	D	ON		TNB		AM	
		year					
		2021	2022	2021	2022	2021	2022
G	0	4.52 ± 0.10^{aA}	5.41 ± 0.73^{aB}	6.20 ± 0.31^{aA}	6.41 ± 0.56^{aA}	5.40 ± 0.29^{aA}	5.59 ± 0.91^{aB}
	20	2.10 ± 0.22^{aA}	3.28 ± 1.32^{aB}	0.30 ± 0.29^{aA}	4.11 ± 2.20^{aB}	2.60 ± 0.33^{aA}	3.26 ± 0.57^{aB}
A	0	15.70 ± 3.61^{bA}	18.27 ± 1.87^{bB}	11.30 ± 3.35^{bA}	15.90 ± 1.73^{bB}	11.70 ± 2.45^{bA}	17.57 ± 3.25^{bB}
	20	7.60 ± 1.51^{bA}	9.40 ± 1.76^{bB}	5.50 ± 0.64^{bA}	6.03 ± 1.22^{bB}	5.70 ± 1.45^{bA}	9.10 ± 2.30^{bB}
F	0	8.30 ± 1.40^{cA}	13.30 ± 1.34^{cB}	23.50 ± 5.01^{cA}	35.57 ± 6.00^{cB}	23.70 ± 5.35^{cA}	35.40 ± 5.20^{cB}
	20	4.20 ± 0.43^{cA}	4.05 ± 1.37^{cA}	11.50 ± 2.01^{cA}	5.33 ± 0.66^{cB}	11.90 ± 1.91^{cA}	5.24 ± 1.21^{cB}
C	0	3.30 ± 0.55^{dA}	5.97 ± 1.61^{dB}	2.80 ± 0.79^{dA}	6.40 ± 0.36^{dB}	3.90 ± 0.71^{dA}	5.13 ± 1.15^{dB}
	20	1.80 ± 0.55^{dA}	1.83 ± 0.94^{dA}	1.30 ± 0.37^{dA}	3.30 ± 0.36^{dB}	1.70 ± 0.45^{dA}	2.13 ± 0.45^{dB}

Legend: S – sample, D – depth (cm), 0 – 0-20 cm, 20 – 20-40 cm, G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil, ON – oligonitrophiles, TNB – total number of bacteria, AM – ammonifiers; a, b, c, d – values from the different sample at same depth for the same parameter and year marked with different letters are significantly ($p<0.05$) different, ANOVA post hoc Tuckey's test. A, B – values from the same sample at same depth for the same parameter and different year marked with different letters are significantly ($p<0.05$) different, T-test.

An abundance of microbes and enzyme activity showed variation depending on management practice, time and depth of sampling (Tables 2 and 3). In C, the significantly lowest value of bacterial number was registered in both years. Upadhyay *et al*. (2016) reported the poor microbial activity of coal mining sites in northern India. Ma *et al*. (2019) showed that coal mine-affected soil characteristics and processes have a strong impact on the soil microbial count. In both years, bacterial abundance were significantly lower in G compared with A and F (Table 2).

Although some studies suggest that low-intensity practices stimulate microbial diversity (Sünnemann *et al*. 2021) in grasslands compared to agricultural soils (Kamgan Nkuekam *et al*. 2018), Romdhane *et al.* (2022) reported the lowest microbial diversity in perennial grasslands. TNB and AM were significantly highest in the F sample, which may be associated with high humus and available potassium content in this sample. Galieva *et al*. (2018) found a high microbial abundance in organic matter-rich soils, which is corroborated by our results. In most of the samples, statistically higher bacterial abundance was observed in 2022. Mencel *et al.* (2022) mentioned that the highest

microbial prevalence occured during vegetation period, which may be associated with the intensive root development and stimulation of microbial growth. In addition, Fierer *et al*. (2003) showed the decrease of microbial prevalence with increasing soil depth, which is confirmed in our study.

Table 3. Actinomycetal abundance and dehydrogenase activity in soil samples

S	D	ACT year 2021 (x 10^4 CFU/g)	2022	DHA 2021 (x10^5 µg TPF/g/h)	2022
G	0	19.0 ± 5.56^{aA}	26.2 ± 8.15^{aB}	1.76 ± 0.18^{aA}	1.28 ± 0.24^{aB}
	20	10.0 ± 4.35^{aA}	14.4 ± 5.78^{aB}	1.43 ± 0.22^{aA}	0.98 ± 0.16^{aB}
A	0	15.0 ± 5.95^{bA}	19.5 ± 1.92^{bB}	2.99 ± 0.52^{bA}	2.27 ± 0.36^{bB}
	20	7.0 ± 3.60^{bA}	10.5 ± 2.26^{bB}	1.84 ± 0.28^{bA}	1.06 ± 0.25^{bB}
F	0	10.0 ± 2.64^{cA}	14.5 ± 3.30^{cB}	2.66 ± 0.40^{cA}	1.22 ± 0.30^{cB}
	20	6.0 ± 1.73^{cA}	6.6 ± 1.84^{cA}	1.78 ± 0.20^{bA}	1.06 ± 0.25^{bB}
C	0	3.0 ± 0.57^{dA}	2.8 ± 0.70^{dA}	0.69 ± 0.19^{dA}	0.73 ± 0.16^{dB}
	20	1.2 ± 0.25^{dA}	1.1 ± 0.17^{dB}	0.33 ± 0.21^{cA}	0.34 ± 0.11^{cB}

Legend: S – sample, D – depth (cm), 0 – 0-20 cm, 20 – 20-40 cm, G – grassland, A – arable soil, F – forest soil, C - coal-mine-affected soil, ACT – actinomycetes, DHA – dehydrogenase activity; a, b, c, d – values from the different sample at same depth for the same parameter and year marked with different letters are significantly ($p<0.05$) different, ANOVA post hoc Tuckey's test. A, B – values from the same sample at same depth for the same parameter and different year marked with different letters are significantly ($p<0.05$) different, T-test.

The number of ACT was significantly highest in surface layer of G and A in both years (Table 3). In these samples, higher pH value was detected compared with other samples. Selianin *et al.* (2005) found the higher ACT presence in alkaline soils. The highest value of DHA was detected in A in both years. However, statistically significant decrease of DHA values in 2022 compared with 2021 was observed. Wolinska *et al*. (2015) revealed a positive correlation between dehydrogenase activity and TNB, which differs from our findings. Nevertheless, Kumar *et al.* (2013) pointed out that numerous factors, such are incubation procedure and time of incubation, temperature, soil aeration and moisture, disturbance, presence of pollutants, and management may affect the DHA in natural and mined soils.

CONCLUSIONS

Presented results showed that management practices have had an impact on the chemical properties and microbial abundance of soil. The lowest microbial and enzyme activity was recorded in coal mine-affected soil.

Although the forest soil was characterized by the lowest pH value and available P content, highest humus content compared with other samples stimulated the development of most bacterial groups and DHA. Further research will be focused on the determination of bacterial and fungal taxa in soil samples influenced by various management practices.

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INFLUENCE OF MINERAL FERTILIZERS AND PLANTING DENSITY ON THE GROWTH, DEVELOPMENT AND YIELD OF NARROW-LEAVED LAVENDER (*Lavandula angustifolia* Mill)

SUMMARY

The purpose of the research was to establish the influence of cultivation technologies on the productivity of narrow-leaved lavender. In a two-factor field experiment, the effects of three options for planting density (14.9 thousand plants/ha, 20.0 thousand plants/ha, 28.6 thousand plants/ha) and five options for the main application of mineral fertilizers ($N_{90}P_{90}K_{90}$, $N_{120}P_{120}K_{120}$, $N_{180}P_{180}K_{180}$ and $N_{240}P_{240}K_{240}$) on biometric dimensions, inflorescence yield, essential oil yield were studied. In a one-factor experiment, the influence of the foliar application of mineral fertilizers (N, P, K separately and NPK together) on the productivity of inflorescences and the content of essential oil was studied.

It was established that increasing the density of planting plants increased the productivity of narrow-leaved lavender. In the variants with the highest planting density - 28.6 thousand plants/ha, the yield of freshly picked inflorescences was from 5.51 to 9.48 t/ha, and the yield of essential oil was from 61 to 107 l/ha, depending on the dose of fertilizer application. A positive effect of the application of mineral fertilizers on lavender yield and essential oil yield was revealed. The highest yield of freshly picked inflorescences of 5.49-9.79 t/ha and the yield of essential oil 60-107 l/ha, depending on the density of planting, was obtained with the main application of mineral fertilizers in the dose of

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$N_{180}P_{180}K_{180}$. Foliar application of mineral fertilizers increased the productivity of lavender's inflorescences (N-by 8.1%, P-by 4.4%, K-by 7.4% compared to the control - without foliar treatments), but reduced the content of essential oil (N-by 4.6 ml/kg, P-by 4.5 ml/kg, K-by 2.9 ml/kg). Foliar application of complex fertilizer (NPK) increased the yield of lavender by 9.6%, reducing essential oil content by 2.7 ml/kg. The content of essential oil in dry inflorescences of narrow -leaved lavender among the investigated variants varied from 35.3 to 39.9 ml/kg.

Keywords: freshly picked inflorescences, yield of essential oil, content of essential oil, quality of raw materials

INTRODUCTION

Narrow-leaved lavender is a perennial semi-shrub of the Libosaceae family originating from the Mediterranean (Mihalascu *et al*., 2020; Pokajewicz *et al*., 2022; Svydenko *et al.,* 2022; Nedeltcheva-Antonova *et al*., 2022). It is grown in Europe, Africa, the USA, and Australia. Flowers and essential oil of narrow-leaved lavender are medicinal raw materials. Demand for lavender essential oil is increasing worldwide (Tarakemeh *et al*., 2012; Giray, 2018). The essential oil has sedative, carminative, antiseptic, anti-inflammatory, analgesic, and bactericidal properties. Narrow-leaved lavender is used by the pharmaceutical, cosmetic, perfumery, and food industries due to the wide range of actions of the essential oil (Pokajewicz *et al.,* 2022; Nedeltcheva-Antonova *et al*., 2022).

For traditional cultivation zones, the yield and quality of the obtained raw material of narrow-leaved lavender primarily depend on the varietal characteristics and soil and climatic conditions of cultivation (Pokajewicz *et al*., 2022; Crisan *et al.,* 2023). Its productivity is also significantly affected by cultivation technology, in particular the area of plant nutrition, provision of nutrients (Chrysargyris *et al,* 2016), moisture, and the level of agricultural technology (Crisan *et al*., 2023).

Research conducted in 2017-2019 by romanian scientists (Mihalascu *et al.,* 2020), confirms that the main application of mineral and organic fertilizers increases the productivity of narrow-leaved lavender. Applying 40 t/ha of cattle manure will increase the yield of lavender's essential oil by 70% (Mavandi *et al.,* 2021). Iranian scientists found that the use of humic acids at a concentration of 5000 mg/l and vermicompost at a dose of 7.5 t/ha provided the highest yield of lavender flowers of 2522.17 kg/ha, with an essential oil content of 3.87% and an essential oil yield of 97.61 kg/ha (Sharafabad *et al.,* 2022).

Bulgarian scientists, who studied the effect of foliar application of microfertilizers on narrow-leaved lavender, found that fertilizers applied by the leaves in the budding phase significantly affect the yield of inflorescences and the yield of lavender essential oil. The average growth of inflorescences was from 25.2 to 29.4%, and the essential oil from 40 to 53.3% was higher than the control (Minev, 2020).

Scientists of the Cyprus University of Technology, who studied the effect of different concentrations of potassium on narrow-leaved lavender under hydroponic conditions, found that 300 mg/l is optimal for obtaining an increased content of essential oil in the raw material (Chrysargyris *et al*., 2017). A change in the concentration of phosphorus in the solution significantly affected the growth processes, and a decrease in the concentration of nitrogen below 150 mg/l reduced the content of chlorophyll. Essential oil yield remained unchanged at different levels of phosphorus and nitrogen concentrations (Chrysargyris *et al.,* 2016).

Narrow-leaved lavender is a drought-resistant plant, but in the initial stages, for intensive growth and development of plants, it needs high soil moisture and the provision of nutrients at an optimal level. This culture is usually grown in the Southern part of Ukraine, in particular, in the Crimea and the Kherson region (Svydenko *et al.,* 2022; Kremenchuk *et al.,* 2017). In the central part of the country, only introductory studies were conducted with narrow-leaved lavender until now.

However, given the climate change towards an increase in the average daily air temperature in the Forest-Steppe zone of Ukraine and the positive experience of the rapid northward expansion of closely related species of Mediterranean origin (Fedorchuk, 2008; Pryvedeniuk *et al*., 2019), the prospects for the success of research on the development of a technology for growing lavender under conditions of the central regions of Ukraine are quite obvious.

Therefore, since 2019, the Research Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of NAAS has begun research on improving the elements of the technology for growing narrow-leaved lavender - *Lavandula angustifolia* Mill. to adapt this valuable crop to new soil and climatic conditions.

MATERIAL AND METHODS

The research was conducted in 2019-2022 on the lands of the Research Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of the National Academy of Agrarian Sciences of Ukraine, which is located on the southern outskirts of the village of Berezotocha of the Lubensky District of the Poltava Region in the Eastern Left Bank part of the Forest-Steppe Zone of Ukraine at an altitude of 160 m above sea level, on the second terrace of the left bank of the Sula River. The location is determined by geographic coordinates: 50°50' north latitude and 30°11' east longitude.

The climate of the Forest-Steppe zone of Ukraine is of a transitional nature between the mild climate of Western Europe and the eastern continental climate. It is due to the increased solar radiation stress, the relatively southern location of the territory, as well as the peculiarities of the atmospheric circulation associated with the influence of the Mediterranean Sea and the Atlantic Ocean.

The weather conditions of the study area were as follows: the average annual air temperature was 9,7°C, the annual precipitation on average over the years of the study was 559.9 mm. The highest average monthly air temperature was 21.8°C in June, the lowest in January was -2.6°C. August was the driest, with an average of 16.5 mm of precipitation at an average monthly air temperature of 21.2°C. The highest amount of precipitation fell in May and December, but their amount was unevenly distributed over the years. May 2022 was the wettest, 176.3 mm of precipitation fell in the form of rain at an average monthly air temperature of 12.9°C. In general, the weather conditions of 2019-2022 were favorable for the growth and development of narrow-leaved lavender. (Table. 1).

Table 1. Meteorological conditions of 2019–2022 (The Research Station of Medicinal Plants, Berezotocha, Ukraine)

Climate factors	Years	Month												Average
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Average temperature (°C)	2019	-5.4	-0.6	3.8	10.4	17.3	23.4	19.9	20.4	15.6	10.5	4.1	2.0	**10.1**
	2020	0.2	1.3	6.6	8.9	12.9	22.1	21.2	20.7	17.9	12.4	3.3	1.6	**10.8**
	2021	-2.8	-5.6	1.8	7.6	14.7	20.6	24.2	21.6	13.1	7.6	4.2	-1.5	**8.8**
	2022	-2.3	0.6	1.4	8.5	14.3	20.9	20.0	22.2	12.2	9.7	2.8	-0.7	**9.1**
	Per years	**-2.6**	**-1.1**	**3.4**	**8.9**	**14.8**	**21.8**	**21.3**	**21.2**	**14.7**	**10.1**	**3.6**	**0.4**	**9.7**
														Total
Precipitations (mm)	2019	57.7	28.7	36.4	28.0	63.4	29.5	22.5	7.1	17.3	27.0	39.6	43.1	**400.3**
	2020	36.4	59.3	11.4	18.0	176.3	38.1	67.7	7.9	30.5	20.9	22.4	44.0	**532.9**
	2021	73.8	64.0	26.6	25.0	75.1	75.7	31.0	28.0	62.6	30.1	45.4	83.2	**620.5**
	2022	60.1	18.4	20.7	62.3	21.7	26.4	137.2	22.8	110.3	59.1	58.6	88.1	**685.7**
	Per years	**57.0**	**42.6**	**23.8**	**33.3**	**84.1**	**42.4**	**64.6**	**16.5**	**55.2**	**34.3**	**41.5**	**64.6**	**559.9**

The experimental scheme was planned according to the methods of B.O. Dospehova. considering the specifics of essential oil plants. The selection of plant samples, biometric measurements, and phenological observations were carried out according to the methods of A.I. Brikin. and Advice O.A. The repetition in the experiment is four times, the options' placement is random. The total area of experimental plots is 60 square meters, the area of accounting plots is 42 square meters.

The soil of the experimental field is heavy, low-humus, light loamy chernozem. The content of humus in the soil is average (2.43%), the thickness of the humus horizon is 80-90 cm, the content of easily hydrolyzed nitrogen is low (103.6 mg/kg of soil), the availability of mobile phosphorus is very high (384.4 mg/kg of soil), mobile compounds of potassium - increased (110.4 mg/kg of soil).

The predecessor was pure steam. Before laying out the experimental plot, plowing was carried out to a depth of 28-30 cm in the autumn period, moisture

was closed with heavy harrows in the spring, and soil cultivation was carried out to a depth of 12-14 cm immediately before planting.

The experimental plot of narrow-leaved lavender was planted with 1-year-old seedlings in the third decade of March 2019. Narrow-leaved lavender seedlings were grown from seeds obtained from Spanish populations. Plants were planted with a row width of 1 m, this row width was chosen for the convenience of caring for plantations using small-sized equipment. In a row, plants were planted at 35 cm (14.9 thousand plants/ha), 50 cm (20.0 thousand plants/ha) and 67 cm (28.6 thousand plants/ha).

Before planting the plants, the main application of mineral fertilizer ($N_{16}P_{16}K_{16}$) was carried out in the dose of $N_{90}P_{90}K_{90}$, $N_{120}P_{120}K_{120}$, $N_{180}P_{180}K_{180}$, and $N_{240}P_{240}K_{240}$, the control was the option without fertilizer application. Foliar application of mineral fertilizers was carried out 3 times with an interval of 10 days, the last application was carried out in the phase of the beginning of flowering. Fertilizers used: for nitrogen application – urea (N_{34}), for phosphorus application – Yara Tera KRISTA MAP (P_{61}), for potassium application – Yara Tera KRISTA K PLUS (K_{46}) and for complex application of nitrogen, phosphorus, potassium – Yara Tera KRISTALON SPECIAL ($N_{18}P_{18}K_{18}$). In all variants, the concentration of the working solution was 2% with an application rate of 300 l/ha.

In the first year of cultivation, lavender was irrigated with a narrow-leaf drip irrigation system, and soil moisture in the 0-40 cm layer was maintained at 75-80% of the lowest moisture content. Soil moisture was monitored with tensiometric sensors of the SPG-II type.

Productivity was calculated during the flowering phase of the crop. Drying of freshly picked narrow-leaved lavender inflorescences was carried out by natural drying. The dryers used were equipped with good ventilation, and the air temperature during drying varied between 27-35°C. Inflorescences were spread with a layer of 4-6 cm on racks covered with fabric and the uniformity of the dehydration process was monitored. The drying period lasted 3-4 days. The collected raw materials were dried to a moisture content of 10-12%. In the first year of cultivation (2019), narrow-leaved lavender did not bloom harmoniously, the flowering period was very long, the number of inflorescences was insignificant, therefore it was not of economic value, and yield records were carried out starting from the second year of vegetation (the yield was calculated as the average for 2020-2022). Determination of the content of essential oil in the inflorescences of narrow-leaved lavender was carried out according to the method outlined in the State Pharmacopoeia of Ukraine using a Clevenger apparatus by distilling it with steam for 2 hours, followed by measuring the volume of essential oil in a graduated tube. The content of essential oil was determined in millilitres per kilogram of raw material in terms of absolutely dry raw material.

Statistical analysis of the results of experiment was performed by variance, correlation, and regression methods using the software Statistica, version 6.0 (TIBCO Software Inc.).

RESULTS

Studying the influence of planting density and mineral fertilizers on the biometric dimensions of narrow-leaved lavender, it was established that with an increase in the number of plants per unit area, their height increased, and the diameter of the bush decreased. So, in the version with a density of 14.9 thousand plants/ha, the height of the plants was from 51.6 to 61.4 cm, depending on the dose of fertilizer application, and the diameter of the bush was 74,5 to 91,4 cm. With an increase in the planting density to 20.000 plants/ha, the height of the plants increased slightly and was 52.5-63.9 cm, and the diameter of the bush decreased and was 72.2-90.9 cm. In the version with the highest planting density, the height of the plants was the greatest and was 53.0-65.1 cm, and the diameter of the bush was the smallest and was only 71.2-88.1 cm (Table 2).

It was found that an increase in the dose of the main application of mineral fertilizers contributed to an increase in the height of plants and the diameter of the bush, in the variants without the application of fertilizers, the height of the plants was 51.6-53.0 cm, the diameter of the bush was 71.2-74.5 cm. In the variants with the application of $N_{120}P_{120}K_{120}$ the height of the bush was 58.2-62.2 cm, and the diameter was 85.5-88.3 cm. The highest plant height of 61.4-65.1 cm with the largest diameter of the bush 88.1-91.4 cm was recorded in variants with the introduction of the highest dose of $N_{240}P_{240}K_{240}$ fertilizers.

The productivity of each individual narrow-leaved lavender plant was characterized by the weight of freshly picked inflorescences from one plant. During the calculations, a tendency to increase the weight of freshly picked inflorescences with a decrease in the density of planting plants was noted. In variants with a planting density of 28.6 thousand plants/ha, the weight of freshly picked inflorescences from one plant was the lowest and amounted to 193-332 g/plant. Reducing the density of lavender planting to 20,000 plants/ha slightly increased the weight of inflorescences and it was 203-343 g/plant. The highest weight of freshly picked inflorescences is 207-368 g/plant had plants in variants with the lowest planting density of 14.9 thousand plants/ha.

During the study of the effect of the main application of mineral fertilizers, a tendency to increase the productivity of narrow-leaved lavender plants with an increase in the dose of fertilizer application was revealed. In the variants with an application of fertilizer in a dose of $N_{60}P_{60}K_{60}$, the weight of freshly picked inflorescences from one plant was 217-253 g/plant. Increasing the dose of fertilizer application to $N_{120}P_{120}K_{120}$ increased the weight of inflorescences to 306-368 g/plant. The highest weight of freshly picked inflorescences is 332-368 g/plant had plants grown in variants with the application of fertilizers in the dose of $N_{180}P_{180}K_{180}$, further increase in the dose of fertilizers was ineffective. In

variants without fertilization, the weight of freshly picked inflorescences was 193-207 g/plant.

Table 2. The effect of planting density and the main application of mineral fertilizers on the biometric parameters of narrow-leaved lavender (average for 2020-2022)

Variants		Height, cm	Diameter of bush, cm	Weight of freshly picked inflorescences of 1 plant, g
Factor A	Factor B			
14.9 thousand plants/ha (100x67 cm)	N_0 P_0 K_0	51.6 ±0.21a	74.5±0.14a	207 ±0,34 a
	N_{60} P_{60} K_{60}	53.8 ±0.04a	82.3 ±0.21b	253 ±0.42b
	N_{120} P_{120} K_{120}	58.2 ±0.01b	88,3 ±0.13b	329 ±1.15c
	N_{180} P_{180} K_{180}	60.3±0,11b	91.3 ±0.19c	368 ±0.89c
	N_{240} P_{240} K_{240}	61.4±0.11b	91,4 ±0.32c	351 ±1.34c
20.0 thousand plants/ha (100x50 cm)	N_0 P_0 K_0	52.5 ±0.04a	72.2 ±0.09a	203 ±1.02a
	N_{60} P_{60} K_{60}	56.9 ±0.12b	81.8±0.14b	230 ±0.76b
	N_{120} P_{120} K_{120}	61.5 ±0.05b	85.8 ±0.02b	324 ±1.25c
	N_{180} P_{180} K_{180}	63.1 ±0.25c	90.5 ±1.17c	343 ±1.09c
	N_{240} P_{240} K_{240}	63.9 ±0.07c	90,9 ±1.27c	331±2.19c
28.6 thousand plants/ha (100x35 cm)	N_0 P_0 K_0	53.0 ±0.02a	71.2 ±0.09a	193 ±0.34a
	N_{60} P_{60} K_{60}	57.5±0.09b	77.4 ±0.13a	217 ±0.49b
	N_{120} P_{120} K_{120}	62.2 ±0.12c	85.5 ±0.19b	306 ±0.97c
	N_{180} P_{180} K_{180}	64.4 ±0.03c	87.8 ±0.07b	332±0.78c
	N_{240} P_{240} K_{240}	65.1 ±0.01c	88.1 ±0.02b	326±1.57c
$LSD_{0,5}$ main effects f. A		1.87	1.34	18.6
$LSD_{0,5}$ main effects f. B		1.53	1.12	17.8
$LSD_{0,5}$ partial differences f. A		2.27	2.01	20.1
$LSD_{0,5}$ partial differences f. B		2.44	2.11	19.4

Note. The difference between the averages (for each parameter) under the different plants density and fertilizers application marked by not the same letter (a, b, c) are significant ($p < 0.05$); average value ± SD

The obtained results prove that with an increase in the density of planting narrow-leaved lavender, the productivity of inflorescences and the yield of essential oil per unit area increased. Thus, in the variants with the lowest planting density of 14.9 thousand plants/ha, the productivity of freshly picked inflorescences ranged from 3.08 t/ha to 5.49 t/ha, depending on the dose of fertilizer application. An increase in the planting density to 20.000 plants/ha

contributed to a rise in freshly picked inflorescences yield to 4.12-6.85 t/ha. The highest yield of freshly picked narrow-leaved lavender inflorescences – 5.51-9.79 t/ha - was obtained in variants with the highest density of 28.6 thousand plants/ha. The dependence of the yield of dry inflorescences on the density of planting is similar to the yield of freshly picked inflorescences (Table 3).

Table 3. The influence of planting density and the main application of mineral fertilizers on the productivity of narrow-leaved lavender (average for 2020-2022)

Variants		Yield of freshly picked inflorescences, t/ha	Yield of essential oil, l/ha	Yield of dry inflorescences, t/ha
FactorA	Factor B			
14.9 thousand plants/ha (100x67 cm)	N_0 P_0 K_0	3.08 ±0.67a	34 ±1.12a	0.99 ±0.66a
	N_{60} P_{60} K_{60}	3.24±0.35a	36±0.03a	1.02 ±0.25a
	N_{120} P_{120} K_{120}	4.90 ±0.02a	54 ±0.87a	1.59 ±0.02a
	N_{180} P_{180} K_{180}	5.49 ±0.12b	60 ±2.78ab	1.75 ±0.29b
	N_{240} P_{240} K_{240}	5.23±0.27b	57 ±0.01a	1.69 ±0.34b
20.0 thousand plants/ha (100x50 cm)	N_0 P_0 K_0	4.12 ±0.89a	45 ±2.24a	1.31 ±0.05a
	N_{60} P_{60} K_{60}	4.59 ±0.11a	50 ±1.18a	1.48 ±0.01a
	N_{120} P_{120} K_{120}	6.72 ±0.22b	74 ±1.17b	2.16 ±0.17b
	N_{180} P_{180} K_{180}	6.85 ±0.04b	75 ±0.09b	2.26 ±0.02bc
	N_{240} P_{240} K_{240}	6.52 ±0.38b	71 ±2.34b	2.13 ±0.04b
28.6 thousand plants/ha (100x35 cm)	N_0 P_0 K_0	5.51 ±1.76b	61±0.47ab	1.75 ±0.17b
	N_{60} P_{60} K_{60}	7.24 ±1.62c	79 ±1.94b	2.29 ±1.56 c
	N_{120} P_{120} K_{120}	9.28 ±0.57c	102±2.35c	2.99 ±1.09c
	N_{180} P_{180} K_{180}	9.79 ±0.12c	107 ±0.58c	3.14 ±0.62c
	N_{240} P_{240} K_{240}	9.48 ±0.44c	104 ±2.02c	2.98 ±0.84c
$LSD_{0,5}$ main effects f. A		0.89	4.65	0.14
$LSD_{0,5}$ main effects f. B		0.72	4.48	0.11
$LSD_{0,5}$ partial differences f. A		1.17	6.65	0.19
$LSD_{0,5}$ partial differences f. B		1.06	6.32	0.16

Note. The difference between the averages (for each parameter) under the different plants density and fertilizers application marked by not the same letter (a, b, c) are significant ($p < 0.05$); average value ± SD

The yield of essential oil depended on the productivity of narrow-leaved lavender inflorescences, in variants with the lowest density of 14.9 thousand plants/ha of plants, it was the lowest and ranged from 34 to 60 l/ha. An increase in the planting density to 20.000 plants/ha contributed to an increase in the yield

of essential oil up to 45-75 l/ha, depending on the dose of fertilizer application. The highest yield of essential oil of 61-107 l/ha was obtained in variants with the highest density of cultivation – 28.6 thousand plants/ha.

The effectiveness of the main application of mineral fertilizers for the cultivation of narrow-leaved lavender was quite high, as during the first year of vegetation, drip irrigation was used to eliminate the soil moisture deficit. Plants effectively used nutrients supplied with mineral fertilizers, as a result of which the intensity of their growth and development partially depended on the dose of fertilizer application. With an increase in the dose of the main application of fertilizers, the biometric dimensions of the plants and their productivity increased. So, in the options with the main application of fertilizer in the dose of $N_{60}P_{60}K_{60}$, the yield of freshly picked lavender flowers was from 3.24 to 7.24 t/ha, depending on the planting density. Increasing the fertilizer application rate to $N_{120}P_{120}K_{120}$ helped to increase the productivity of narrow-leaved lavender in variants with such a dose of fertilizer application, the yield of freshly picked inflorescences was 4.90-9.28 t/ha. The most effective fertilizer application dose was $N_{180}P_{180}K_{180}$, where the productivity ranged from 5.49 to 9.79 t/ha of raw materials, further increasing the fertilizer application dose was less effective. In variants without fertilization, the yield of freshly picked narrow-leaved lavender inflorescences was the lowest and ranged from 3.08 to 5.51 t/ha, depending on the planting density.

The use of mineral fertilizers helped to increase the productivity of narrow-leaved lavender and, at the same time, increased the yield of essential oil, so in the variants without applying fertilizers, the yield of essential oil was from 34 to 60 l/ha, depending on the density of planting plants. In variants with the application of fertilizers in the dose of $N_{60}P_{60}K_{60}$, the yield of essential oil was 36-79 l/ha. Increasing the dose of fertilizer application to $N_{120}P_{120}K_{120}$ contributed to an increase in the yield of essential oil to 54-102 l/ha. The highest yield of essential oil of 60-107 l/ha was obtained in variants with an application of fertilizers in the dose of $N_{180}P_{180}K_{180}$, further increase in the dose of application of fertilizers was less effective. During the study of the complex effect of planting density and the dose of mineral fertilizers on the productivity of narrow-leaved lavender, the lowest yield of freshly picked inflorescences of 3.08 t/ha with the lowest yield of essential oil of 34 l/ha was obtained in the variant with the lowest density of 14.9 thousand plants/ha without application of fertilizers The highest indicators of 9.79 t/ha of freshly picked inflorescences and 107 l/ha of essential oil were obtained at the highest density of 28.6 thousand plants/ha and with the introduction of mineral fertilizers in the dose of $N_{180}P_{180}K_{180}$.

The dependence of the yield of narrow-leaved lavender essential oil on the application of mineral fertilizers can be described mathematically by the following equations:

$$y = -2.9025x2 + 26.214x + 20.499, R^2 = 0.93,$$

where: y – yield of essential oil, l/ha,
x – dose of mineral fertilizers, kg/ha of active substance,
R^2 is the value of the reliability of the approximation.

The value of the reliability of the approximation is 0.93, which indicates the high reliability of the dependence of the essential oil of narrow-leaved lavender on the dose of mineral fertilizers (Figure 1).

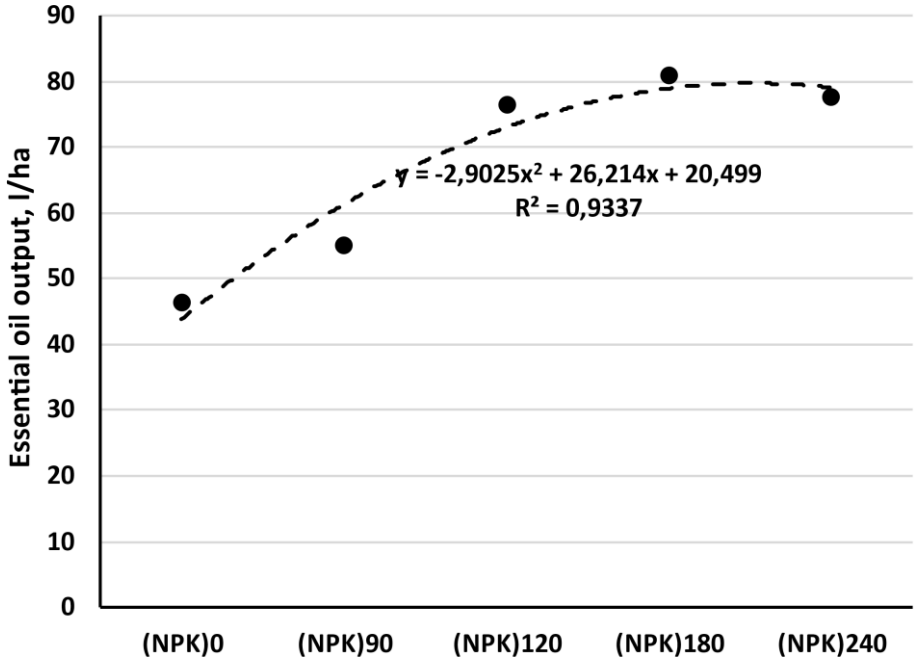

Figure 1. Dependence of the of narrow-leaved lavender essential oil output on the dose of mineral fertilizers

The main quality criterion of dry inflorescences of narrow-leaved lavender, which is used by the pharmaceutical industry for the manufacture of medicinal products, is the content of essential oil.

The influence of the foliar application of mineral fertilizers on the productivity and content of essential oil in the inflorescences of narrow-leaved lavender was studied. The obtained results indicate that three times foliar applications of nitrogen fertilizer increased the productivity of inflorescences by 8.1% compared to the control, but at the same time reduced the essential oil content by 4.6 ml/kg compared to the control.

The use of fertilizer with a high phosphorus content increased the yield of inflorescences by 4.4%, reducing the content of essential oil by 4.5 ml/kg compared to the control. Foliar application of potassium increased the yield of dry lavender flowers by 7,4%, reducing the content of essential oil by 2.9 ml/kg.

Foliar application of a complex mineral fertilizer increased the yield of lavender by 9.6%, and the raw material obtained in this variant contained 2.7 ml/kg less essential oil compared to the control. On the control variant, dry inflorescences of narrow-leaved lavender contained 39.9 ml/kg of essential oil (Figures 2, 3).

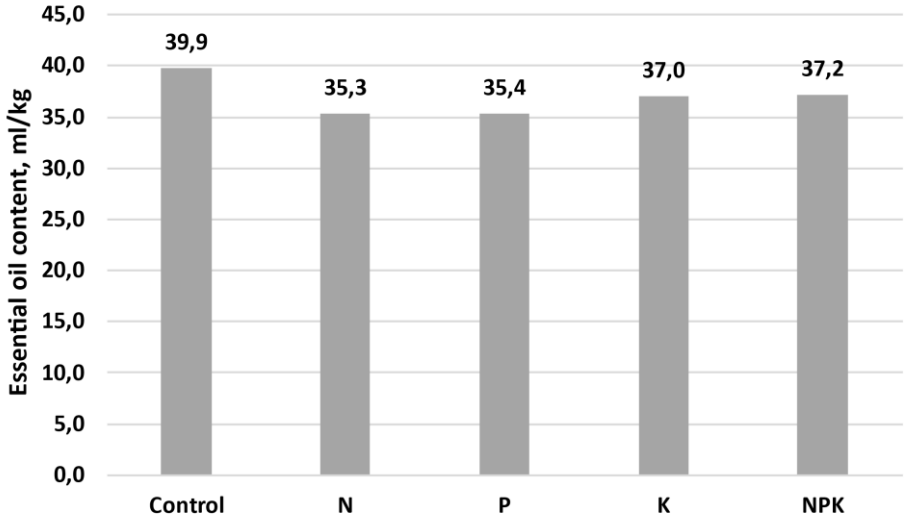

Figure 2. Effect of foliar application of mineral fertilizers on the essential oil' content in dry inflorescences of narrow-leaved lavender

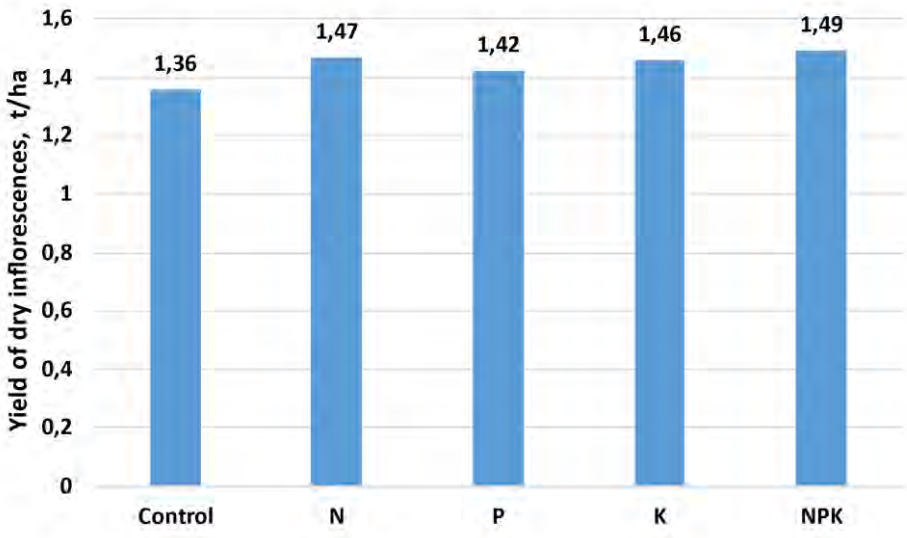

Figure 3. The effect of foliar application of mineral fertilizers on the dry inflorescences' productivity of narrow-leaved lavender.

Therefore, when the foliar application of mineral fertilizers, it is necessary to take into account the fact that with the increase in the yield of dry inflorescences of narrow-leaved lavender, the content of essential oil decreases somewhat.

According to the requirements of the European Pharmacopoeia and the State Pharmacopoeia of Ukraine, dry raw materials (inflorescences) of narrow-leaved lavender must contain at least 13 ml/kg, calculated on anhydrous raw materials. The obtained results indicate that in all studied variants of narrow-leaved lavender raw materials, the essential oil content indicators were 2.5-3.0 times higher relative to the minimum current requirements, which indicates the possibility of obtaining high-quality lavender raw materials in the production conditions of the Left Bank Forest Steppe of Ukraine.

DISCUSSION

Changes in weather conditions that have been observed in recent decades allow for revising the distribution zones of a number of essential oil crops, which until recently were considered suitable for cultivation only in the southern regions (Svydenko, *et al*., 2022). Among the crops that are rapidly expanding the area of cultivation, is the narrow-leaved lavender *Lavandula angustifolia* Mill. occupies a leading position (Svydenko *et al*., 2022; Kremenchuk *et al*., 2017; Rudnik-Ivashchenko *et al.,* 2018). Studies conducted in different soil and climatic conditions prove the prospects of this culture in the conditions of the Forest-Steppe zone of Ukraine (Kremenchuk *et al.,* 2017; Rudnik-Ivashchenko *et al.,* 2018). However, the highest productivity and product quality can be achieved by optimizing the growth and development of plants at the main stages of the formation of yield elements, which is convincingly proven by several studies on the study of medicinal and essential oil crops (Pryvedeniuk *et al*., 2021; Shatkovskyi *et al*., 2021). As research results show, at each stage of development, plants need specific ratios of environmental conditions, and the closer they are to the optimum, the better conditions are created for the formation of high plant productivity, especially for new growing areas (Pryvedeniuk *et al,* 2021).

The average annual rainfall during the growing season (April-October) of our research was 372 mm, which is sufficient for the intensive growth and development of narrow-leaved lavender. The obtained results indicate that increasing the density of planting lavender plants increased the productivity of inflorescences and the yield of essential oil in the first three years of harvesting. We obtained the lowest yield in the variants with a planting density of 14.9 thousand plants/ha, and the highest - in the variants with a planting density of 28.6 thousand plants/ha. A similar trend is observed with narrow-leaved lavender, an increase in planting density contributed to an increase in the yield of inflorescences, according to studies by Turkish scientists. Among the studied options, they obtained the highest yield of lavender flowers for the 30x30 cm cultivation scheme (111 thousand plants/ha) (Ceylan *et al,* 1996).

According to scientific sources, the use of fertilizers on narrow-leaved lavender has a positive effect on its growth, development and productivity (Chrysargyris *et al*., 2018; Crisan *et al*., 2023; Mavandi *et al.,* 2021; Sharafabad *et al.,* 2022; Elshorbagy *et al.,* 2020; Biesiada *et al.,* 2008). Providing lavender plants with nutrients due to the main application of fertilizers helps to increase the productivity of the crop. Studies by Brazilian scientists prove the high efficiency of organo-mineral fertilizers application to lavender increasing the dose of applying fertilizers increased the productivity of the culture (Silva *et al.,* 2017). Similar results were obtained by Egyptian scientists using mineral fertilizers, they got the highest productivity of lavender with the introduction of the largest amount of nitrogen, phosphorus, and potassium among the studied options (Ceylan *et al*., 1996). The results of our research confirm the high efficiency of using mineral fertilizers when growing narrow-leaved lavender. Increasing the dose of nitrogen, phosphorus, and potassium before planting lavender contributed to more intensive growth and development of plants. In variants with high doses of fertilization, plants had larger biometric sizes compared to the control and formed high yields of inflorescences and essential oil.

The obtained research results show that foliar application of nutrients increases the yield of lavender flowers, but at the same time slightly reduces the content of essential oil. This is explained by the fact that the mass of plants began to increase more intensively due to the introduction of an additional source of nutrients, but the accumulation of essential oil was not so rapid, there was an effect of reducing the concentration of essential oil due to the increase in biomass. Studies by Bulgarian scientists with foliar application of fertilizers indicate that they can increase and decrease the yield of lavender essential oil (Minev *et al.,* 2022). Therefore, when applying foliar fertilizers, you should strictly follow the recommendations, especially if fertilizers are applied before harvesting.

Based on the experience of domestic and foreign scientists, as well as on the results of own research with medicinal and essential oil crops, developing a system of agrotechnical measures for growing narrow-leaved lavender L*avandula angustifolia* Mill. in the conditions of the Left Bank Forest-Steppe of Ukraine, the peculiarities of the culture and new soil and climatic conditions for it were taken into account to create an optimal growing environment and obtain high productivity indicators.

CONCLUSIONS

According to the results of the research, the optimal level of mineral nutrition and planting density of narrow-leaved lavender plants were established. According to the yield criteria of inflorescences (9.79 t/ha) and essential oil (107 l/ha), the best option for mineral nutrition was the application of a dose of fertilizer $N_{180}P_{180}K_{180}$, and the best option for planting plants was a 100x35 cm scheme with a density of 28.6 thousand/ha.

The dependence of the yield of narrow-leaved lavender essential oil on the level of mineral nutrition of plants has been proven. The established dependence is described by a mathematical equation -y = -2.9025x2 + 26.214x + 20.499, and the approximation reliability value is 0.93, which indicates a high degree of reliability of the dependence of essential oil yield on the level of mineral nutrition.

The regularities of the influence of foliar top dressing with mineral fertilizers and complex fertilizers (NPK) on the yield of inflorescences and essential oil of *Lavender angustifolia* have been investigated and established. In particular, foliar top dressing with mineral fertilizers increased the yield of inflorescences (N-by 8.1%, P-by 4.4%, K-by 7.4% compared to the control - without foliar top dressing), but reduced the content of essential oil (N-by 4.6 ml/kg, P-by 4.5 ml/kg, K-by 2.9 ml/kg). Foliar application with complex fertilizer (NPK) increased the yield of inflorescences by 9.6%, reducing the content of essential oil by 2.7 ml/kg.

The generalized result of the research proved the possibility of forming a relatively high level of productivity of plantations of angustifolia lavender by optimizing mineral nutrition and planting density in the conditions of the Forest-Steppe zone of Ukraine.

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EFFECT OF LATITUDE ON SUGAR BEET BOLTING OCCURRENCE

SUMMARY

Sugar is one of the sources of energy supply in the country's basic commodity basket. The two distinguish crops namely sugar cane and sugar beet are used for sugar production in Iran. Although sugar beet is mainly grown as a spring crop but the autumn-sown sugar beet cultivation is growing to overcome water shortage. In this study, the effect of latitude on bolting occurrence in four research stations, with wide latitude difference, during the period of 2011 to 2018 was evaluated. Data were collected from 58 value for cultivation and use (VCU) trials including registered cultivars and advanced breeding material. Results showed that the highest bolting percentage was recorded in regions with high latitude including Moqan followed by Mashhad. Beside the results, it could be observed that other factors such as number of cold days during winter and especially the planting date may influence bolting occurrence. To the best of our knowledge, this is the first study evaluating the effect of different latitudes on sugar beet bolting percentage in Iran.

Keywords: autumn planting, bolting, latitude, sugar beet

INTRODUCTION

Iran is one of the countries in the world that, in terms of climate diversity, in addition to producing sugar from sugar beet and sugar cane, it is possible to plant sugar beet in both spring (in cold temperate regions) and autumn (in tropical and warm temperate regions). Currently, due to limited water resources, it is not possible to develop spring sugar beet cultivation. The most important indicators of priority and superiority of autumn-sown sugar beet cultivation compared with

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spring cultivation are the use of rainfall during the growth period, higher yield, fewer pests and diseases, and especially less water demand and high water use efficiency. This issue becomes doubly important because the lack of water resources is the most important challenge and limiting factor in the production of agricultural products in the country (Fasahat *et al*., 2018; Saeedi and Ziaee, 2020). The development of autumn-sown sugar beet cultivation and the supply of a part of sugar beet needed by sugar factories in areas that are facing limited water resources can be very important in the sustainability of sugar beet production in those areas. The autumn cultivation of sugar beet in Iran was initially started in Dezful region, and many studies have been conducted on various aspects of agronomy, quality and other characteristics of autumn-sown sugar beet during the past years. Results of studies confirm that autumn sugar beet can be introduced as an important crop in the rotation system of susceptible areas (Orazizadeh *et al*. 2020). Currently, the most important challenge in spring crops is the lack of water resources and the uncertainty of supplying the amount and time of water needed by the plant. This problem is gradually developing in different regions, and even in regions that did not have problems in this regard before, it is becoming the main limitation in the production of agricultural products. Although in the future due to the escalation of water shortage, there is a possibility of reducing the area under cultivation of spring crops, including spring sugar beet, but in recent years, by changing the geography of production, sugar factories supply their sugar beet from areas with less drought restrictions. Thereby, the share of eastern regions in the country in sugar beet production decreased from 47.6% to 24.2% and the share of western regions in sugar beet production increased from 21% to 41.2% (Fasahat *et al*., 2020; Taleghani *et al.,* 2020; Rezaei and Fasahat, 2022).

With all the advantages that autumn-sown sugar beet cultivation has over spring one, one of the major limitations for autumn cultivation is the bolting occurrence. Sugar beet is a biennial plant and the incidence of frost in winter followed by long days in spring causes the sugar beet to bolt and flower (Milford and Burks, 2010). In addition to severe reduction in final yield via reduction in sugar content and root yield, the bolting occurrence in early plant growth stage may also disturb sugar beet harvester, slowing down the cutting and slicing blades in the sugar factory due to hardening and fibrosis root shape and increasing the probability of seed scattering (cause the growth of sugar beet as weed in following years) (Longden and Scott, 1980; Longden, 1989). As a result, bolted sugar beet plants are not suitable for sugar production, and these sugar beets are only dry products with a high percentage of shoot and a small amount of root. Some concerns such as water use efficiency, long growing season of sugar beet followed by sequential drought and water shortage especially in the southern regions of Iran has highlighted the vital role of autumn-sown sugar beet cultivation for sugar supply. With the above-mentioned information, in this study, for the first time the effect of latitude on sugar beet bolting occurrence in main locations of the country that are considered for autumn-sown sugar beet cultivation was evaluated.

MATERIAL AND METHODS

Data collection

In this study, data were taken from 3464 observations based on plots from 58 VCU trials performed in four research stations across Iran during the period of 2011 to 2018. The data set comprised 121 diverse sugar beet genotypes including registered cultivars and advanced breeding material and from 8 harvest years. Within one year and location up to 3 different trials were available, however mostly only one trial was grown. The geographic area represented includes Mashhad (Torbat-e-Jam), Moqan (Parsabad), Dezful and Shiraz (Darab) where most of the autumn-sown sugar beet cultivation is done (Fig. 1).

Fig. 1. Location of four research stations in Iran

General characteristics of the trial locations such as longitude/latitude, the average temperature and rainfall during the studied period are presented in Table 1 and 2.

Table 1. Characteristics of the trial locations

No.	Location	Longitude	Latitude	Sea level (m)	No. of trials	No. of observations (plots)
1	Moqan (Parsabad)	47°46'E	39°36'N	67	8	392
2	Mashhad (Torbat-e-Jam)	60°48'E	35°12'N	838	14	800
3	Dezful	48°24'E	32°21'N	121	22	1344
4	Shiraz (Darab)	54°33'E	28°45'N	1185	14	928

Climatically, the Moqan location is expected to have a colder climate than the location Shiraz. However, the high altitude of Shiraz compared with Moqan has moderated the temperature conditions in this location compared with its latitude.

Table 2. Characteristics of the temperature and rainfall during the period of 2011 to 2018

Location	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Moqan (Parsabad)												
Temp-Max (ºC)	17.96	19.13	23.73	27.08	32.28	37.23	38.48	37.69	33.60	28.00	20.38	16.95
Temp-Min (ºC)	-6.64	-4.20	-1.01	2.58	10.23	14.61	17.00	16.54	12.04	6.14	-0.46	-3.34
Average monthly temperature	5.66	7.46	11.36	14.83	21.25	25.92	27.74	27.11	22.82	17.07	9.96	6.81
Monthly rainfall (mm)	22.08	28.52	24.21	14.34	27.00	24.65	5.38	4.12	25.83	44.39	21.58	18.08
Seasonal rainfall (mm)		74.81			65.98			35.32			84.04	
Mashhad (Torbat-e-Jam)												
Temp-Max (ºC)	18.58	19.73	26.58	31.26	37.04	39.40	40.20	38.16	35.05	31.85	24.31	19.61
Temp-Min (ºC)	-8.08	-7.66	-2.44	3.75	10.99	15.26	17.85	14.01	10.06	1.79	-3.46	-8.83
Average monthly temperature	5.25	6.03	12.07	17.51	24.01	27.33	29.03	26.09	22.56	16.82	10.43	5.39
Monthly rainfall (mm)	14.03	29.09	22.76	17.93	11.15	0.04	0.00	0.10	0.00	5.19	14.04	6.99
Seasonal rainfall (mm)		65.88			29.12			0.10			26.22	
Dezful												
Temp-Max (ºC)	22.59	25.54	30.49	37.79	44.44	48.00	50.56	48.58	44.88	39.55	30.09	24.18
Temp-Min (ºC)	1.81	3.25	6.54	9.43	16.95	19.96	23.50	23.35	19.09	13.35	8.16	3.03
Average monthly temperature	12.20	14.39	18.51	23.61	30.69	33.98	37.03	35.96	31.98	26.45	19.13	13.60
Monthly rainfall (mm)	44.18	21.29	33.06	15.34	16.07	0.00	0.00	0.25	0.25	11.25	50.26	37.02
Seasonal rainfall (mm)		98.53			31.42			0.50			98.53	
Shiraz (Darab)												
Temp-Max (ºC)	22.48	23.31	27.11	33.54	39.76	43.21	43.86	42.81	39.90	35.25	27.88	24.01
Temp-Min (ºC)	-0.35	1.50	4.24	9.39	15.44	17.93	22.75	20.99	16.58	10.71	5.15	0.23
Average monthly temperature	11.06	12.41	15.68	21.46	27.60	30.57	33.31	31.90	28.24	22.98	16.51	12.12
Monthly rainfall (mm)	45.25	85.55	41.52	9.67	2.30	0.05	6.19	2.31	0.74	0.38	30.56	10.67
Seasonal rainfall (mm)		172.31			12.02			9.24			41.61	

Each VCU trial was conducted in a randomized complete block design with four replications. Based on the number of entries, 2-4 check cultivars were added to the trial. Each plot had three 8 m rows and counting of the bolted plants

was performed at the plot level. The bolting percentage was calculated as the number of plants that bolted in each plot divided by the number of all plants × 100. The trials were managed according to local agronomic practices.

The following model was used:

$$y_{ijklr}=\mu+G_i+Y_j+L_k+(YL)_{jk}+(YL/T)_{jkl}+(YL/T/B)_{jklr}+(GY)_{ij}+(GL)_{ik}+(GYL)_{ijk}+\varepsilon_{ijklrh}$$

where y_{ijkrh} represents the percentage of bolting plants in the ijklrhth plot, G_i is the effect of the ith genotype, Y_j of the jth (harvest) year, L_k of the kth location, $(YL/T)_{jkl}$ of the lth trial within year j and location k, $(YL/T/B)_{ijklr}$ of the rth block within trial l of year j and location k, $(GY)_{ij}$ the interaction between genotype i and year j, $(GL)_{ik}$ the interaction between genotype i and location k, $(GYL)_{ijk}$ is the interaction between genotype, year and location and ε_{ijklrh} is the residual error. The main effects for locations L_k and μ were considered as fixed, while the other effects were assumed to be random and independent with constant variance for each effect.

RESULTS AND DISCUSSION

In the present study, the average bolting percentage during the studied period was 15.06% with minimum and maximum of zero and 100, respectively (Fig. 2). As it has been shown in Table 3, the effect of Mashhad, Shiraz, and Moqan locations on bolting percentage was significant. The highest bolting percentage was observed in Moqan (36.67%) followed by Mashhad (28.82%), Shiraz (13.77%), and Dezful (1.47%), respectively (Fig. 2, 3). The long-term meteorological data show that the average monthly minimum temperature of Moqan and Mashhad decreases to less than zero during winter, in Dezful it was above zero and falls below zero only in the coldest month of the year (January) in Shiraz location (Table 2). The average maximum monthly temperature in Moqan location during July and August was lower than Mashhad, Dezful and Shiraz locations (Table 2). In total, the average annual temperature of Moqan, Mashhad, Dezful and Shiraz locations were 16.5, 16.8, 24.7 and 21.9°C, respectively (Table 2). The long-term average of total annual rainfall in location Moqan was more than all locations, however the seasonal distribution of rainfall in Mashhad was more balanced than other locations.

Table 3. The effect of location on bolting percentage and standard error comparison among locations

Location	Bolting (%)		
	Mean	SE	*P* value
Mashhad	28.82^{b}	7.18	0.0001
Shiraz	13.77^{c}	8.91	0.0312
Moqan	36.67^{a}	10.15	0.0003
Dezful	1.47^{d}	7.06	0.9459

n.s. = not significant; Least square means with the same letter are not significantly different at the 0.05 probability level. P >|t| is the probability that the mean is greater

than 0.

The pairwise tests for the least squares means of bolting percentage in studied locations showed that only Dezful had significant ($p < 0.05$) difference with Mashhad and Moqan and no difference was observed among other locations (Table 4).

Table 4. Pairwise tests for the difference between least squares means of bolting percentage for studied locations

Location	Dezful	Moqan	Shiraz
Mashhad	0.0023	0.6248	0.3002
Shiraz	0.0986	0.1849	
Moqan	0.0030		

n.s. = not significant, P >|t| is the probability that the mean is greater than 0.

The means, medians, quartiles, maximums and minimums of observations based on plots from VCU trials are presented in Fig. 2.

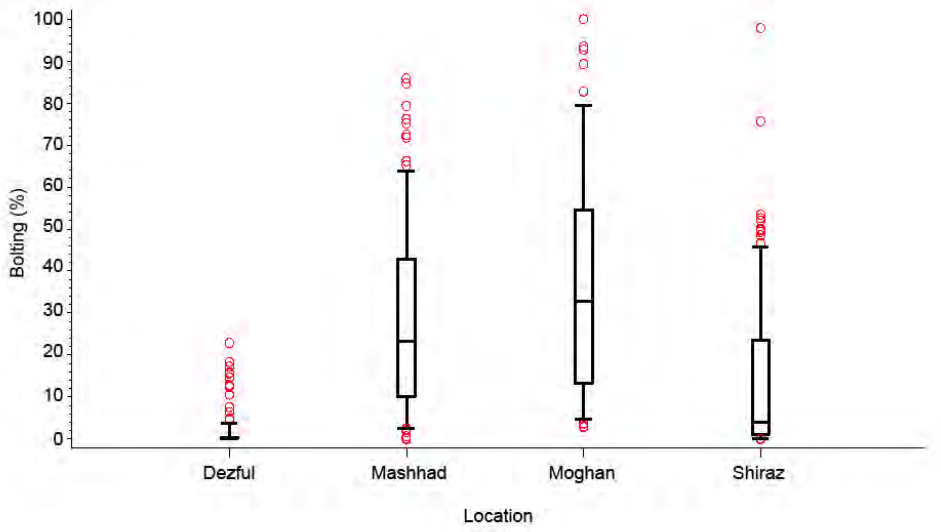

Fig. 2 Means and variation of bolting percentage from VCU trials performed in four locations during the period of 2011 to 2018.

Sugar beet is considered as a crop of temperate locations with main area of cultivation being between 30° and 60° N in Asia, Europe, and North America (Cooke and Scott 1993); however, studies showed that the maximum yield can be achieved at latitudes between 30° and 40° and decrease swiftly between 40° and 55° (Loomis and Gerakis, 1973). This has also influenced the life cycle of wild beets of the genus Beta (*B. vulgaris* ssp. *vulgaris*) so that under Mediterranean climate (southern latitudes), they exhibit an annual life cycle without need of vernalization, while sugar beets (*ssp. vulgaris*) and wild beets in northern

latitudes are mainly biennial and require vernalization for flower induction (Letschert, 1993; Van Dijk *et al*., 1997; Boudry *et al.* 2002; Melzer *et al*. 2014). In addition, Hoft *et al*. (2018) reported later flowering of accessions from northern latitudes than those from southern latitudes. Kuroda *et al*. (2019) explained the high frequency of annual haplotypes among sugar beets in Japan as a result of relatively low latitude compared with Unites States and Europe.

Fig.3. Photos taken from different locations including a) Dezful (by Dr. Hasanvandi), b) Mashhad (by Dr. Rezaei), c) Moqan, and d) Shiraz (by Dr. Bazrafshan)

In this study, the latitude of Moqan is high which stimulates the bolting occurrence. In a study by Farahmand *et al.* (2013), the possibility of autumn-sown sugar beet cultivation in Moqan location was evaluated through three planting dates including 23rd September, 12th October, and 1st November with two cultivars susceptible and resistant to bolting. Their results showed that the effect of cultivar on bolting was significant ($P < 0.01$). The bolting percentage was strongly affected by planting date and with the delay in planting, the rate was reduced so that the average of bolting percentage in susceptible and resistant cultivars was 82% and 5%, respectively.

Same condition was observed for Mashhad. As it could be seen in Table 2, Mashhad followed by Moqan experienced more cold days/temperatures than Dezful and Shiraz which stimulates bolting occurrence. In a study by Mohamad Yosef *et al.* (2016), 47 half-sib families of sugar beet with three controls were evaluated at Torogh station (36°12'N and 59°40'E and 985 m above sea level) in Mashhad. Results showed that the effect of genotype on bolting percentage was significant ($P < 0.01$) with an average bolting percentage of 69.7 in a range of 0-97.62%.

The number of cold days in Dezful are less which is not enough for bolting induction. In contrast, Shiraz experience cold days during winter and long days in spring which provides suitable condition for bolting occurrence. The correlation

between the number of cumulative hours of vernalization and the bolting percentage showed that with increasing the total cumulative hours of vernalization, the bolting percentage increases. This relationship was not statistically significant from the time of planting to 100 days old, and then the linear significant relationship between the number of hours of cumulative vernalization with the bolting percentage ($p<0.05$) and from the thirteenth week after (planting) to the end of the growing season ($p<0.05$) was reported (Taleghani *et al*., 2013). Therefore, it can be inferred that a significant difference between bolting percentage in the years and also different planting dates is due to the low total vernalization temperature received by the plants. For example, in Dezful region, due to the low total number of vernalization hours received, the bolting percentage varied between 0-70%. In a three-year study by Ashraf-Mansouri *et al.* (2013), three sowing dates including 27th September, 17th October, and 6th November was evaluated in Fasa location (28°58′N and Longitude 53°41′E with 1300 m elevation above sea-level) in 2005-08.

Results showed that sowing on 27th September led to the highest bolting (18.4%) whereas 17th October and 6th November had the lowest bolting by 5.420% and 2.870%, respectively. Year had significant effect ($p<0.01$) on root yield and bolting percentage. Since early sowing in locations with resembling condition to Fasa may subjected plants to longer cold weather, it contributes to the augment of the number of bolted plants. However, in later sowing, this period is shorter and as a result, the bolting percentage decreases. Van Dijk *et al.* (1997) averred that flowering time in southern parts is controlled by day length and warm temperatures. Riihimäki and Savolainen (2004) reported same condition for southern populations of *Arabidopsis lyrata* compared with northern ones in all environmental conditions including a common garden experiment and in the field. Therefore, sensitivity to vernalization is geographically structured so that the southern accessions are more sensitive to vernalization duration than northern ones (Stinchcombe *et al.* 2005). In a study by Yoshie (2014), the genetic difference in bud formation time was evaluated in *Taraxacum officinale* plants grown at three different latitudes (26, 36, and 43°N). It was shown that at high temperatures, the inhibition of flower bud formation follows a descending trend with latitude from north to south.

CONCLUSIONS

Due to the gradual warming of the earth, in the future it is anticipated that the autumn cultivation of sugar beet may replace the spring cultivation. Studies on the mechanism of flowering in plants show that the bolting stimulation is mainly due to factors such as day length, vernalization, and internal physiological factors of the plant such as size or growth rate (which itself can be related to environmental factors such as temperature). In this study, evaluation of bolting occurrence in different latitudes showed that the trend was ascending in accordance with latitudes with exception of Dezful in where the cold days' number was few.

The breeding programs for producing tolerant cultivars to bolting is in progress in different countries however, cultivar adaptability to latitudes as well as planting dates are main factors to be considered.

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NETWORK REAL TIME KINEMATIC (CORS-FKP METHOD) ACCURACY IN/UNDER FOREST AREA

SUMMARY

The use of Global navigation satellite system (GNSS), (geolocation and navigation system) in/under forestry is increasingly being established, especially in the areas of logistics, inventory and measurements. However, the uninitialized forest user assumes that GNSS will always work with the same accuracy everywhere, while the more critical foresters assume that GNSS will probably not work properly in/under the forest. A criterion is often used to evaluate the performance of a GNSS receiver in an obstructed environment. Due to tree canopy in a forest, GNSS performance can be limited, disturbed or completely prevented. The Real Time Kinematics (RTK) approach can provide centimetre-level accuracy in a suitable environment. A more accurate and stable positioning technique was first proposed in the mid-1990s by meticulously modelling all fault components based on a known reference network called the Network Based RTK (NRTK) technique. Since then, several methods have been developed and implemented in practice, such as the Virtual Reference Station (VRS), the Flächen Korrektur Parameter (FKP) and the Master-Auxiliary Concept (MAC), which are fundamentally similar approaches. The purpose of all studies for this paper was to evaluate the achievable accuracy of the Continuously Operating Reference Stations (CORS)-FKP in the project area and check the results. The CORS-FKP results show that the solution for the forest area was reached at cm level (±10-15 cm) for horizontal accuracy.

Keywords: Global Navigation Satellite System; gps; real time network; precision; static

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INTRODUCTION

Satellite navigation systems are becoming a significant tool in many professions. Global Navigation Satellite System (GNSS) refers to all available navigation satellite systems, GPS (Global Positioning System) and GLONASS (GLobal NAvigation Satellite System), Galileo and Beidou (Compass). It also includes newer systems such as China's BDS (BeiDou Navigation Satellite System), Japan's QZSS (Quasi-Zenith Satellite System), India's IRNSS (Indian Regional Navigation Satellite System), and Europe's Galileo (ESA 2016), (European GNSS (Galileo) Open Service 2021). (Wolf and Ghilani 2002).These satellite systems are sent into orbit and transmit coded electromagnetic signals back to Earth. A GNSS device can calculate its position in space and time by observing and analyzing signals from many satellites. Although the system has various features, in ecology, GNSS devices are usually used to locate bug traps, plant measurement plots, soil boring test locations, tree ring age measurement locations, and species occurrences (Konnestadt 2018). Because GNSS requires continuous connectivity to navigation satellites, using GNSS in semi-open environments produces unexpected results. Trunks, branches, or tree tops may block, reflect, or otherwise disrupt the GNSS signal, and this problem is predicted to worsen as forest density increases. The usage of GNSS beneath forest canopy is a regular aspect of the mapping workflow in many circumstances. This mapping may fail to provide the correct information for natural resource management if the difficulties with signal degradation in forest areas are not addressed. It may also be difficult to examine these coordinates because they do not give any information on how far it is predicted to be from its genuine position (Konnestad 2018, Kaplan 1996, Bakula *et al*. 2015, Brach and Zasada 2014, Frank *et al*. 2014, Massimiliano 2018, Gilbert 2002, Hofmann-Wellenhof *et al.,* 2001, Cina *et al*. 2015, Koivula *et al*. 2018, Pehlivan *et al*. 2019).

Continuously Operating Reference Stations (CORS) networks have been widely employed for high-precision real-time location. The availability of widely dispersed GNSS CORS networks prompted the implementation of the NRTK approach, which allows stations to overcome distance limitations (Wolf and Ghilani 2002), (Rizos 2002), (Shuanggen 2012). (Prochniewicz *et al*. 2020). The utilization of the GNSS CORS network also enables for more reliable differential corrections across large regions, such as the Virtual Reference Station (VRS) technique (Wanninger, 2003), (Mageed, 2013) the Multi Reference Station (MRS) approach (Fotopoulus *et al.* 2001), the Flächen Korrektur Parameter (FKP) approach or other surface correction approaches (Keenan *et al*., 2002), (Kim *et al*., 2017). Several authors (Prochniewicz *et al*., 2020), (Kim *et al*., 2017), (Dobelis *et al*. 2016) demonstrated that the NRTK technique allows reaching centimeter accuracy, comparable with the accuracy of the static measurements. FKP (area correction parameter) was developed by Geo++ Company, and its detailed information is given by (Wübbena *et al*. 2001, Wübbena and Bagge 2002, Cina *et al*. 2015). The correction parameters are calculated based on the number of surfaces estimated for each CORS station and the calculation of the correction parameters; the changes in the north-south and east-west directions are defined. For each reference station, a unique FKP surface

is estimated. This data will allow it to converge quickly and accurately. The biggest problems in working with GNSS in the forest and wooded areas are the inability to see enough satellites and the decreases in the strength of the signals received from the satellites seen. As a result of the decrease in the signals of the satellites, the distance between the satellite and the receiver cannot be measured accurately. This causes problems in terms of accuracy in calculating point coordinates (Hofmann-Wellenhof *et al.,* 2001, Koivula *et al*. 2018).

The aim of this study is to evaluate the FKP method's performance in/under the forest environment. The purpose of all experiments in this paper was to investigate the achievable accuracy of the CORS-FKP in the project area and check the obtained results.

MATERIAL AND METHODS

Study Area

To investigate these problems; the effects of forest area that are associated with CORS-FKP positioning, three experiments (CORS-FKP, static, total station surveys) were carried out in the Davutpaşa region (Yıldız Technical University Campus), near Istanbul, in Turkey. For this aim, (K5, K6, K7 and K8) stations were marked in the project area (see Figures 1 and 2). K5, K6, K7 points were located under/in the forest area; but K8 point was marked in the unobstructed area (Figures 1 and 2). The area of interest is distinguished by its location on the border of a wooded region and an open sky. Data distortion and signal losses impair GNSS receiver observations in forestry, reducing precision and accuracy. To avoid this, CORS FKP surveys have been done on the open area side of the border with the goal of giving precise observations to improve the GNSS receiver's communications with satellites. The points whose coordinates are being calculated was established at the forested area side, and CORS FKP surveys and static GNSS surveys were also conducted (Pirti 2010). The data collection and processing speed for CORS-FKP surveys was set to 1 second and 5 epochs with a 10 degree elevation mask angle. The integer ambiguity is fixed between 1 minutes and 35 minutes for each point on 30 September 2020 and 1 October 2020 by using Topcon Hiper HR receiver. The first survey was performed on 30 September 2020 by using FKP technique whereas the other survey was performed on the successive day (1 October 2020) by using FKP technique.

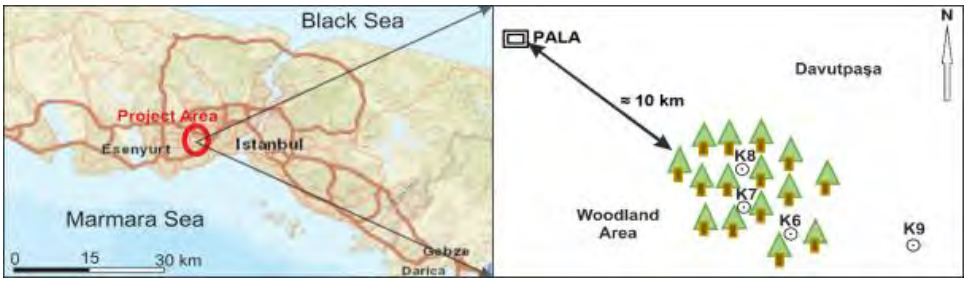

Figure 1. Project area and GNSS network

Figure 2. The four points (K6, K7, and K8 under the forest area and K9 in the unobstructed environment) in the study area

Description of the experiments

On October 2, 2020, static GNSS measurements of these four points were observed for at least 2.5 hours. The data receiving and processing rate is set to 30 seconds, and the cut-off elevation mask angle is set to 10 degrees. By using ISKI-CORS reference station PALA (approximately 10 kilometres away from the project), about 2.5 hours of GNSS static measurement values were calculated at three points (in/below the forest area, see Figure 2 and Figure 3). Area) and commercial post-processing GNSS Software, Topcon magnet tool (version 5.1.1.0). During the adjustment process, the ITRF 2005 coordinates of ISKI CORS/PALA points are fixed (Figure 1, Table 1). Table 1 and Table 2 respectively show the coordinates and standard deviations of the four points, as well as the date and time of observation. The GNSS equipment used for CORS measurement consists of a pair of Topcon Hiper HR receivers (Static (Horizontal =3mm+0.1ppm, Vertical=3.5mm+0.4ppm)), (RTK (Horizontal=5mm+0.5ppm, Vertical=10mm+0.8ppm)), (Topcon Manuals 2020). The two tests by using ISKI-CORS FKP surveys were performed at different times on two days (30 September 2020 and 1 October 2020), see (Table 2). The number and distribution of GNSS satellites tracked were generally "normal", 7 to 13 satellites were observed (Figure 4), and Position Precision Dilution (PDOP) ranged from 1.5 to 3.8, see Fig. Table 2 (Pirti 2010).

Table 1. Standard deviation and coordinate values of the four points by using static surveys

Point	Grid Northing (m)	Grid Easting (m)	Elevation (m)	Std N (mm)	Std E (mm)	Std h (mm)
PALA	4550678.029	412882.003	170.573	0	0	0
K6	4543679.333	406841.909	103.132	3	3	4
K7	4543678.792	406822.821	103.148	5	5	7
K8	4543669.766	406808.598	104.004	4	4	5
K9	4543657.721	406756.410	105.513	3	3	4

Figure 3. Sky visibility from Points K6, K7 and K8 (a, b, c), respectively

As explained above, GNSS receivers of K6, K7 and K8 were situated under/in the area; see Fig. 3. The problem shown by the sky plot of 9:20-9:39 hours on 30 September 2020 (Fig. 4a) and 10:18-10:26 hours on 1 October 2020 (Fig. 4b) is typical for the whole day; several satellites were shaded by the trees, see Fig. 4. As can be seen from the skyplot (Fig. 4), the receiver tracked satellites, at a high/medium elevation in the obstructed areas of the sky. Nine satellites were shaded in the obstructed area. Strong signal distortion may therefore be expected because these nine satellites have low elevations at this period for K6, K7, and K8 on two days. The signal scatter and signal attenuation is partially due to the low elevation. This effect occurs due to multipath caused by trees environment, see Fig. 3 and Fig. 4.

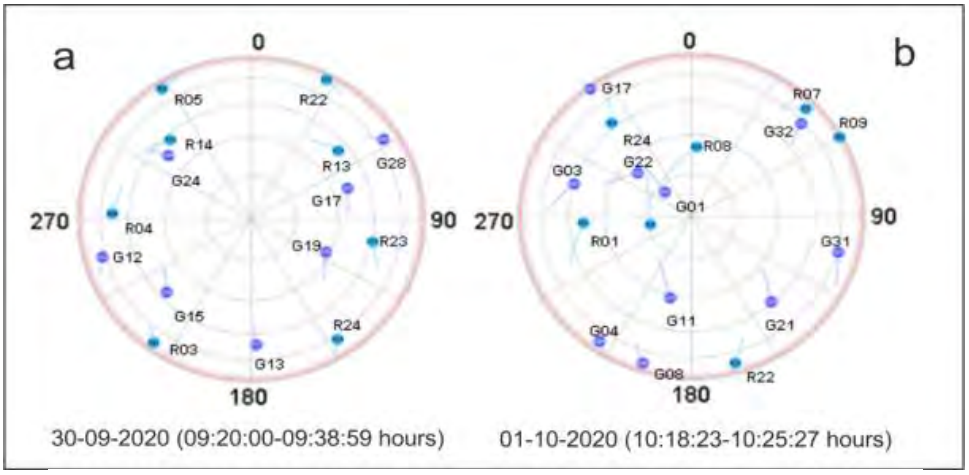

Figure 4. The sky plots in the project area on 30 September 2020 and (a) 1 October 2020 (b)

Table 2. Time schedule of the two FKP measurements for four points by using Topcon Hiper HR receiver

Test 1 Test 1 DateTime Datum	Point Točka	Easting Istočno	CORS-FKP Northing Sjeverno	El.Hgt.	Ep.	Hz	SAT.	hRms	vRms	Pdop	Method	Status
2020-09-30-09:32:03	**K6**	406822.708	4543678.710	102.480	5	1	9	0.006	0.009	3.756	FKP	FIX
2020-09-30-09:36:20	**K7**	406841.916	4543679.295	103.025	5	1	7	0.005	0.006	2.275	FKP	FIX
2020-09-30-09:38:59	**K8**	406756.377	4543657.668	105.567	5	1	13	0.005	0.006	1.706	FKP	FIX
2020-09-30-09:20:00	**K9**	406808.583	4543669.729	103.958	5	1	9	0.004	0.006	3.109	FKP	FIX
Test 2			**CORS-FKP**									
DateTime	**Point**	**Easting**	**Northing**	**El.Hgt.**	**Ep.**	**Hz**	**SAT.**	**hRms**	**vRms**	**Pdop**	**Method**	**Status**
2020-10-01-10:22:16	**K6**	406822.809	4543678.780	103.176	5	1	12	0.004	0.006	1.588	FKP	FIX
2020-10-01-10:23:10	**K7**	406841.800	4543679.397	103.789	5	1	12	0.006	0.008	2.295	FKP	FIX
2020-10-01-10:25:27	**K8**	406756.413	4543657.672	105.507	5	1	13	0.005	0.007	1.564	FKP	FIX
2020-10-01-10:18:23	**K9**	406808.619	4543669.767	103.902	5	1	10	0.004	0.006	2.929	FKP	FIX

RESULTS AND DISCUSSION

Although FKP Technique is less used in the world than VRS Technique today, it is preferred as a result of the developments in real-time-position determination studies. In this study, the accuracy and repeatability analysis of the FKP technique was examined. The performance, accuracy and sensitivity values of the FKP technique in the clear and forested areas were calculated by using the coordinates obtained at different times. While the internal accuracy values of FKP were obtained using measurements made at different times, the external accuracy value was calculated by comparing the values obtained with static measurements. The purpose of the first experiment is to check CORS-FKP and evaluate its performance in forest areas/under forests. The experiment involves a set of four points (K6, K7, K8, and K9) marked on the ground. (Please note that the survey was conducted in different satellite constellations and at different times of the day (see Table 2)). Figure 5 shows the coordinate difference between the FKP measurement results of the four points. Figure 5 also shows the average and standard deviation of the coordinate differences obtained from the first and second CORS-FKP measurements of the four points. Comparing the measurement results, the horizontal coordinates of the points determined by these tests appear to be the same, but there are some changes between a few millimetres and 12 centimetres. However, the consistency of the height component is poor, and sometimes the maximum change amplitude at the same point between two CORS-FKP sessions is 76 cm, as shown in Figure 5. The standard deviation of the horizontal coordinate differences was about 10 cm. The standard deviation of height differences was about 50 cm, see Fig. 5. The mean value of horizontal component is about 6-7 cm and the mean value of height component is about 40 cm (Pirti 2010).

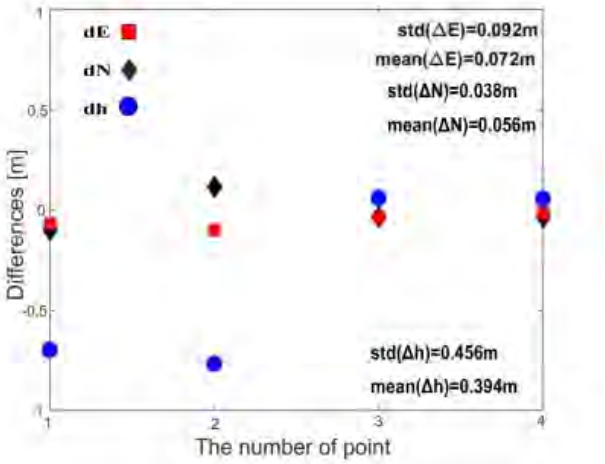

Figure 5. Comparison of the coordinates obtained from FKP surveys for four points on 30 September 2020 and 1 October 2020

The obtained coordinates of the static GNSS survey for four points are compared with the two FKP survey results of four points (on 30 September 2020 and 1 October 2020), see Fig. 6 and Fig. 7, respectively. By September 30, 2020, the coordinates (east and north) of the four points are usually sufficient, the standard deviation value is less than 5 cm, and the average value is less than 6 cm. Between static GNSS measurement and CORS-FKP measurement, the height components at the same height are not consistent, sometimes the difference is about 70 cm. The standard deviation and mean values of height of four points were 30 cm and 22 cm, respectively, see Fig. 6.

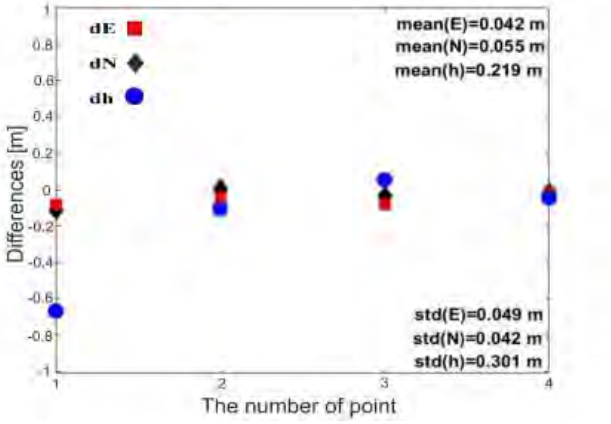

Figure 6. Comparison four points coordinates by using FKP with static coordinates on 30 September 2020

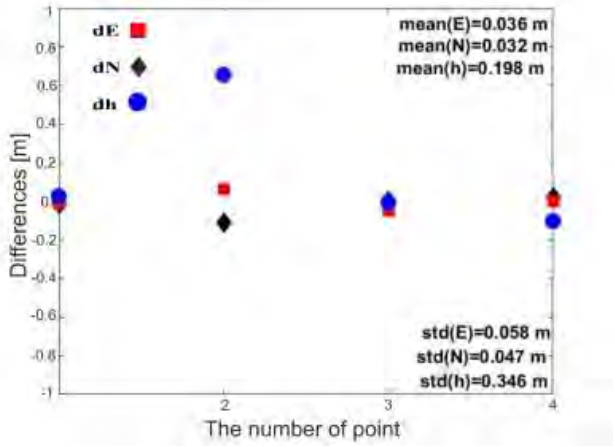

Figure 7. Comparison four points coordinates by using FKP with static coordinates on 1 October 2020

By using CORS-FKP measurement and static GNSS measurement, the coordinate difference (east, north) between the four points by October 1, 2020 is usually large enough, the standard deviation value is less than 6 cm, and the average value is less than 4 cm. Between static GNSS measurement and FKP measurement, the height components at the same height are not consistent, sometimes the difference is about 70 cm. The standard deviation and average height of the four points are 35 cm and 20 cm, respectively, as shown in Figure 7.

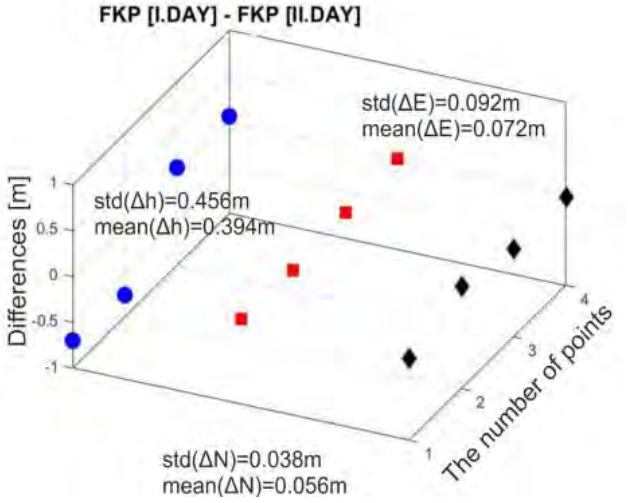

Figure 8. Comparison of the four points coordinates obtained from CORS-FKP surveys on 30 September 2020 (I. Day) and 1 October 2020 (II. Day)

Figure 8 shows two tests (FKP [I.DAY (September 30, 2020)] – FKP [II.DAY (October 1, 2020)] in the east, north, and altitude (Up) coordinate directions. The mean and standard deviation values of coordinates of all points (eastward, northward) are usually very good, and the standard deviation is less than 10 cm; as expected, since the average standard deviation reaches about 50 cm, the height accuracy is less than this value, and the height The weight is smaller at the same time point between two CORS-FKP treatments, sometimes even as long as 76 cm (Figure 8).

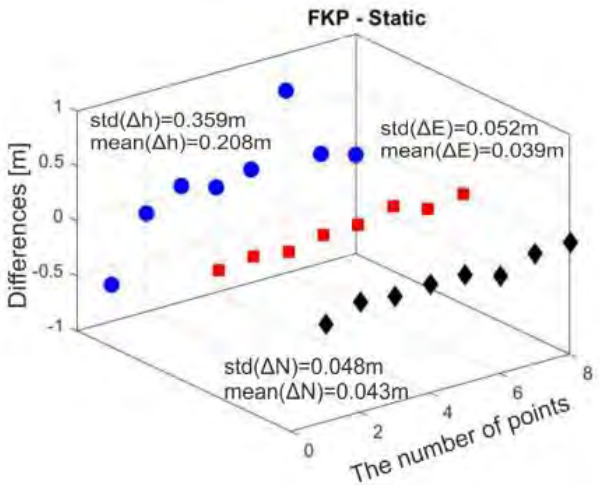

Figure 9. Compare all of the coordinates of the points by using static GNSS method and CORS-FKP method

Figure 9 shows the coordinate difference between CORS-FKP and static GNSS measurement results. Fig. 9 also shows the average value and standard deviation of the coordinate difference. When comparing the results of the two methods, the horizontal coordinates determined using these tests, respectively, appear to be consistent with changes ranging from a few centimetres to 11 centimetres. However, the consistency of the height components is poor, and sometimes the difference between the static GNSS and CORS-FKP heights at the same point is about 70 cm. The horizontal coordinates of the four points (between the CORS-FKP measurement and the static measurement) determined by these tests look very consistent, varying from a few centimetres to 11 centimetres. However, depending on the influence of obstacles in the field in the project area (forest canopy), the consistency of the height component is not very good, and the change is small, sometimes up to 70 cm. All results also show that forest canopy impair the positioning of CORS-FKP. Therefore, even with good satellite windows, signal blockage from forest canopy can be seen as the main problem when using CORS-FKP in the blocked area.

The points in the forest are obscured from decent satellite views. The FKP approach is also ineffective when satellite signals are low or absent due to

extensive tree canopy or stems, among other things. This research demonstrates that the FKP survey may be utilized for woodland or forest surveys (obtaining precision to the cm-dm level), despite a common impediment, sky blocking, limiting its full usefulness. However, if traditional survey methodologies are used in addition, this difficulty can be solved. The CORS-RTK (FKP) took around 30 minutes to survey a site in this investigation. The horizontal plane coordinates of the sites (beneath the trees) varied by up to 10-15 cm. As a result, it appears that in tough conditions (forest), readings of 1 cm or less cannot be assured in all cases when employing the FKP technique. In the future, I would want to measure additional locations in the forest and test the accuracy of GNSS (GPS/GLONASS/Galileo/BeiDou) positioning utilizing CORS FKP/VRS techniques. The obtained results in this study are consistent with those of many other groups that made similar tests. The horizontal and vertical accuracy in obstructed areas discussed in this paper are in agreement with those of the other authors (Andersen *et al.* 2009, Bakula *et al.* 2015, Brach and Zasada 2014, Dobelis and Zvirgzds 2016, Frank and Wing 2014, Wing and Eklund 2007, Kaartinen *et al.* 2015, Keenan *et al*. 2002, Kim *et al.* 2017, Mageed 2013, Massimiliano 2018, Naesset and Gievestad 2008).

CONCLUSIONS

As a result of the measurements performed with FKP, the values of the horizontal coordinate differences obtained in terms of repeatability and accuracy remained in the range of 10-15 centimetres. However, the coordinate differences in height values are calculated in the range of 40-50 centimetres. The horizontal coordinate differences between the two-day FKP measurements and the static measurements were found in the range of 3-6 centimetres. Coordinate differences in height values were obtained in the range of 20-35 centimetres. FKP method, this modelling it is limited, the rover can only use data from two stations to compute the atmospheric model.

Forest cover has some negative effects on GNSS signals, such as blocking, attenuation and reflection. The results of this study show that CORS-FKP can achieve a horizontal accuracy of 10-15 cm and a vertical accuracy of 75 cm in forest areas (points K6, K7, and K8). These results show that CORS-FKP is suitable for positioning and other applications that will not cause adverse conditions in blocked areas. However, CORS-FKP can obtain a horizontal accuracy of 1-4 cm and a vertical accuracy of 5-10 cm in an unobstructed area (Point K9). Due to the low accuracy of FKP correction in large-area modelling deviation, it is not suitable for positioning even in a medium-scale network. However, when analysing the 2D and 3D components together, the static technique shows better results than the FKP technique. Particularly where the centimeter level horizontal and vertical accuracy is required, the CORS RTK method is problem free. This study shows that the CORS FKP method can replace other survey methods in the forest applications which require the above mentioned accuracy.

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AN OVERVIEW OF LIVESTOCK BREEDING IN THE MEDIEVAL ZETA THROUGH THE LEGAL NORMS OF THE STATUTES OF KOTOR, BUDVA AND SKADAR

SUMMARY

The statutes of the medieval Zeta communes (Kotor, Budva, Skadar) also contain norms that, in whole or in part, also refer to animal husbandry. In the area of city districts, livestock could be kept on private properties, but also on municipal properties. One of the measures adopted to ensure the best possible conditions for cattle breeding was the ban on the surrounding population grazing their cattle in the area of the district. In the same way, the communal authorities adopted measures concerning the regulation of the use of certain lands as pasture areas in the area of the district. Apart from own management, the livestock could be raised by giving it to graze to other persons who took care of the livestock. Cattle were raised primarily for the household's needs, food, the sale of surplus agricultural products and their use for cultivating the land and transporting goods. Apart from this benefit from raising cattle, its owners could have certain incomes and benefits, as can be seen from the statutory provisions, from renting out draft and cargo cattle. In addition to rent, livestock was also used as a means of pledge, as stated in the statutes in Budva and Kotor. Livestock raised in Zeta communes caused damage to agricultural crops, and the communal authorities prescribed norms that provided for sanctions for the damage caused. Analyzing those sanctions from the norms of the Kotor legal system, it can be noted that cargo and draft animals, that is, their owners, were sometimes more leniently, or differently, sanctioned for the same damage than other livestock, while their killing was not allowed.

Keywords: statutes, animal husbandry, the Middle Ages

INTRODUCTION

The economy of the people in the medieval Zeta communes was determined above all by social conditions, but also by the natural environment that existed in them. Social conditions were generally more dominant than natural conditions for economic development. This resulted in the dynamic development

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of trade, crafts, fishing, and shipping as important economic branches in those communes. Due to the physio-geographic characteristics of the area, the natural conditions for cattle breeding were not very favorable (Djurović P. and Djurović M. 2015; Marković *et al.* 2017; Barović *et al*. 2020). And yet, a significant part of the population in the communes was engaged in agricultural activities, animal husbandry, or farming.

Medieval communes consisted of a town, which usually had a suburb and a district. The district was a wider area governed by a legal regime enacted by the authorities of the commune that governed it. Today, to a certain extent, it is possible to reconstruct the approximate area occupied by the districts of Zeta communes in the Middle Ages (Antonović, 2003). From these data, it can be seen that part of those city territories was an area suitable for agriculture, that is, animal husbandry. Over time, the need arose in the communes to settle issues related to animal husbandry, in addition to other issues. In addition to the norms that directly concerned that activity, it is indirectly discussed in some other legal norms, primarily those concerning the cultivation of land and the damage caused to agricultural crops by cattle. Those norms, direct and indirect, give answers to some questions that talk about the livestock farming of the population of Zeta communes, as well as the importance of this activity in the economy of Zeta communes. This paper aims to present and analyze the legal norms from the preserved statutes of Zeta communes that relate to animal husbandry and to consider its importance in the economy of Zeta communes, which until now has not been the subject of detailed analysis and study in science. Animal husbandry in Montenegro today represents the most important branch of animal breeding and agriculture in general (Adžić *et al.* 2004; Marković *et al.* 2021).

MATERIAL AND METHODS

The material for writing this paper can be divided into several categories. The core material used to write this paper are the medieval statutes of Kotor, Budva and Shkodra, whose texts have been preserved and published (Statuta Civitatis Cathari, 1616; Statuta *et* leges civitatis Buduae, 1988; Statuta di Scutari 2016).

In addition to written norms, in the Zeta communes, when it comes to animal husbandry, customary law was also applied, which would represent the next, although much less common source (material) used for writing this work (On customary law, see: Marinović, 1974; Statute of the City of Kotor II, 2009).

A very significant comparative material for the study of this topic is represented by the statutes of medieval Dalmatian communes, first of all, due to their geographical proximity and similar socio-economic structure, the statute of Korčula (Statuta *et* leges civitatis *et* insulae Curzullae, 1877), but also of other smaller Dalmatian communes, that were close in social structure to Zeta communes, especially Budva and Skadar.

In the statutes of Zeta communes, as the material on which the work was written, one cannot notice the efforts of the organizers to regulate issues related to

animal husbandry in detail, and in one place. This seemed to be the most common legal norming of matters centered on another issue. We could divide the legal norms of the Zeta communes that speak about animal husbandry into several categories. The first would be those few provisions that directly relate to that activity. This category of norms includes those that regulate the use of areas for livestock grazing by the population of the district, that is, they sanction unauthorized use by persons outside the city district, who did not have the right to do so. Norms related to the ways of raising livestock and their use can also be counted there. In them, we come across mentions of persons who had the obligation to look after livestock. The second group consists of those provisions which, indirectly, also testify to livestock farming in the communes. These are mainly regulations that regulate the issue of damage caused by cattle on agricultural properties. The last group of regulations testifying to animal husbandry consists of those in which, through the regulation of some other concerns, some issues that can be linked to animal husbandry are mentioned and thus testify to that activity. Although there are not many legal norms about animal husbandry in the statutes of Zeta cities, they can give a certain picture of animal husbandry in them and its importance for the economy of those communes.

It can be safely assumed that part of the legal norms related to animal husbandry in Zeta communes originates from customary law. Thus, the legal institutes that were in force, and which are said to be regulated according to customary law, are mentioned in the Kotor official and notary documents. The absence of a large number of legal norms on animal husbandry in the statutes of Zeta communes can be interpreted precisely by the application of customary law. It can be assumed that the legal norms found in the statutes reflect the legal awareness and needs of a period, which does not mean that they were not subject to change. In some cases, these changes can be traced to the fact that at some point legal norms related to animal husbandry were replaced or supplemented by others.

When it comes to the methods used to write this paper, analytical and comparative methods are dominant. The material was approached in such a way that, to the extent possible, as far as the statutes of the Zeta communes allowed, individual topics, i.e. issues concerning animal husbandry, were analyzed, with a simultaneous comparison of those solutions, both in the Zeta statutes and those in the statutes of the Dalmatian communes.

RESULTS AND DISCUSSION

We could start the story by showing the area where livestock could be raised in Zeta communes, that is, grazed, and under what conditions. The basis for raising cattle was private land holdings within the district owned by the inhabitants of Zeta communes. We learn about their existence through the norms of all three statutes, which talk about the ownership of private immovable property, which also included pasture land, as well as preserved Kotor archival material. Those properties, in private ownership, were the subject of some norms

in the statutes (Bujuklić 1988; Bogojević Gluščević 1992; Statuta di Scutari 2016). In addition to private estates, areas owned by the communes were of particular importance for livestock farming in the communes. As we will see, the municipal authorities in some cases prescribed the conditions for their use for grazing.

The use of private properties for livestock grazing is directly evidenced by the Kotor Statute norm passed in 1421 by the Venetian authorities. It was stated that the population of Kotor could not graze their cattle to cattle breeders outside the district, i.e. to those from the hinterland, nor to lease their land holdings for grazing, which also applied to foreigners (Statuta Civitatis Cathari, 1616). (In this way, the Venetian authorities in Kotor, in addition to severing ties with the hinterland after Kotor accepted their supreme authority, wanted to preserve pasture areas only for the needs of the population of their commune (Sindik, 1950). This provision says that the population that reared cattle during the summer gave the cattle to shepherds from the surrounding areas for grazing, which in turn speaks of the insufficiency of pasture land in the area of the Kotor district. In question was the well-known custom of Katun cattle herding, widespread in the Middle Ages, but also the modern period (On Katun cattle herding in the Middle Ages see: Symposium 1963; Božić 1968; Luković 2015; Isailović 2017; Marković *et al.* 2018). The lack of pasture land in the area of the Kotor district is also reflected in the charter of Queen Jelena of Anjou for the village of Zator, which is located near Kotor. Under the threat of a large fine, the inhabitants of Kotor were forbidden, among other things, to graze their livestock in the area of that village, the boundaries of which are detailed in the charter (Gogić, 2021). Some similar measures, to secure pasture areas only for their population, were adopted in the 14th century by the Budva authorities. The statutory provision of that commune stipulated that foreigners may not graze on Budva's borders and territories, with the threat of a fine. That ban did not apply to the population from Suzana, probably today's Šušanj (Statuta et leges civitatis Buduae, 1988; Bujuklić 1988). The concern of the Budva authorities about the cattle and their nutrition is reflected in the norm by which the city judges, under the threat of punishment, are obliged to buy all the grass from the places where the vlašts were not settled (Statuta et leges civitatis Buduae, 1988). The statute of the city of Shkodra makes a provision that talks about the existence of pastures and a special regime for their use. This provision does not tell us enough about the ownership o f those pasture areas, but it would rather be said that they are common, i.e. municipal areas than private properties of the Skadar population. According to this norm, the guardians of the pastures were obliged to conscientiously guard the pastures (herbi). If the guard caught someone (slave, Albanian or Skadran) grazing sheep, cows or pigs during the night, he had the right to take one sheep, cow or pig from that person. In addition, he had to inform the court about it, with the testimony of two or three other guards (Statuta di Scutari, 2016). The existence of pasture guards in Skadar speaks of the efforts of the communal authorities to provide pasture areas for the needs of their

population, who had the right to do so. Some Dalmatian communes also adopted norms on the regime of use of municipal pastures, bringing sometimes interesting solutions. Thus, the Korčula statute prescribes the grazing regime in Kneže, the area that was at the disposal of that commune. It was prescribed that during the day anyone could graze livestock in that area (except for kids and lambs), while at night grazing was prohibited in that area. This norm was later changed so that neither goats nor sheep were allowed to graze in that area, neither day nor night (Statuta et leges civitatis et insulae Curzullae, 1877). The Skadar statute does not contain a provision prohibiting the use of pasture areas by the population that did not belong to the district, which does not mean that such measures were not regulated by customary law, bearing in mind the importance of those areas for the economy of the commune's population. Similar measures aimed at banning the use of pasture lands belonging to the commune by the surrounding population, as was done by the authorities of Kotor and Budva, are also stated in the statute of the commune of Brač (Cvitanić, 1968).

All three communes adopted measures aimed at banning or limiting livestock grazing in certain areas within the district, primarily vineyards and areas sown with grain. The Kotor Statute talks about it in detail. When in 1307 the commune of Kotor received the parish of Grbalj from the Serbian king Milutin, and thus expanded its district, its authorities began to organize the legal regime in it. One of the segments of that arrangement referred to the more precise conditions of use of that fertile area. Thus, in the statutory provision created after 1316, it is prescribed that no one may use someone else's sown field for grazing, nor the grass that someone left behind as grassland. Furthermore, in the same provision, it was stated that when the owner of that land with grass starts to use that grass for grazing, then his neighbors can use it for grazing riding horses and oxen for plowing (Statuta civitatis Cathari 1616). An undated provision stipulated that no one should dare to graze horses, mules, donkeys, oxen, cows, pigs and sheep at any time in vineyards, orchards, gardens and sown fields, with detailed sanctions for violators of this provision, o which will be discussed later in the work (Statuta Civitatis Cathari, 1616; Katić, 1978). However, at one point the Kotor authorities deviated from this norm. It was about the fact that the statutory provision from 1406 allowed the grazing of oxen in the vineyards during their plowing, during the day. In the evening, the oxen had to be chased away from the vineyard (Statuta civitatis Cathari 1616; Gogić 2016). The municipality of Skadar prescribed a ban on grazing cows, horses or donkeys within areas planted with wheat before the end of the harvest. Cases were exempted from this decision when the cow was tied and thus could not cause harm (Statuta di Scutari, 2016). In the same commune, by statutory provision, it was forbidden for anyone to graze cows in one part of the district, the area between the church of St. Peter and the church of St. Angel, in the period from spring to the feast of St. Andrea (Statuta di Scutari 2016). Similar measures, banning cattle grazing in a certain area, were adopted by the Korčula authorities. By the norms they brought, it was forbidden to graze cattle in front of the city, excluding horses and donkeys, as

well as cattle for slaughter (Statuta et leges civitatis et insulae Curzullae 1877). Similar to the one in Skadar, this issue was also regulated in the Budva statute. It stated that no one could graze oxen, horses or donkeys between the grain until it was harvested, except if the animals were tied and could not cause harm (Statuta et leges civitatis Buduae, 1988). As can be seen, unlike the Kotor commune, which almost explicitly forbade it, the Budva and Skadar communes allowed cattle grazing on arable land in some situations, and only in those cases when the cattle were tethered. The safety of arable land was their priority. All of the above could, on the one hand, be interpreted as the great concern of the Kotor commune for viticulture, which represented an extremely important branch of agricultural space. This could be considered indirect proof of the importance of animal husbandry in the economy of the population of those communes and the aspirations of their authorities to help their animal husbandry population in this way.

The statutes of Zeta communes give norms about the areas where livestock could be housed, or more precisely, where it was not allowed. Medieval communes were communities where, for the most part, there were no clear boundaries between urban and rural lifestyles. This was also manifested by the fact that the population within the cities themselves, ie. the city walls kept livestock, which the communal authorities tried to prevent. This was also the case in medieval Kotor. From the 13th-14th centuries, there are no legal norms related to the regulation of this issue. From the beginning of the 15th century (1406), there is a provision that prohibited the keeping of cattle in the city. Whoever wanted to keep livestock could still build a stable outside the city of Kotor, specifying two locations near the city, as well as several others a little further from the city, where this could be done (Statuta Civitatis Cathari, 1616; Sindik 1950; Katić 1978). So, while the Korčula authorities tried to prevent livestock from grazing in front of the city, the Kotor commune adopted a norm prohibiting the keeping of livestock in the city. There is no doubt that this problem existed in the Kotor commune before. The adoption of this norm at that moment was possibly conditioned by the consequences of political events in the Kotor area. Those were the years when, under the pressure of armed conflicts, the population from the area of the district, as well as the surrounding areas, sought refuge within the city walls, probably trying to place some of their movable property there. Such a problem is not regulated in the Budva statute, while in Skadar it is mentioned indirectly. It states that pigs cannot be kept in the city, within the norm related to the damage that pigs could do to cultivated areas (Statuta di Scutari 2016). Certainly, such measures were adopted in Kotor and Skadar mainly for hygiene and health reasons. Keeping livestock in the limited space of the city walls could worsen the living conditions of the population inside them and thus potentially be the cause of some infectious diseases.

The next question related to animal husbandry in Zeta communes is the question of how the animals were raised. The first was that the population only raised livestock within their household. It was mainly applied by the population

of the rural areas of the communes, within their households. Another way, also represented, was giving cattle to graze to other people. When it comes to the Kotor statute, there are no provisions in it that regulate this type of livestock farming. Preserved archival materials from Kotor testify that Kotor nobles and citizens gave cattle to the peasants for grazing. Those peasants, therefore, in addition to their own livestock, also took other people's for safekeeping. Keeping other people's livestock was regulated by contracts, which stated that they were concluded according to customary law (secundum consuetudines terrae) (Mayer 1950; Sindik 1950). In contrast to Kotor, the Budva statute contains provisions related to the provision of livestock for grazing and the existence of shepherds who were responsible for this. The first provision (Statuta et leges civitatis Buduae, 1988; Katić, 1978; Bujuklić, 1988) prescribes the method of giving cows or sheep for grazing. Two-thirds of the offspring belonged to the owner of the cattle, and a third to the shepherd. In case of any damage to the animals, which would be interpreted as an omission by the shepherd, he was obliged to compensate it. He was exempted from this only if the theft of the livestock entrusted to him for safekeeping occurred during the night, provided that he informed the owner of the livestock about the theft in time. Another provision regulates the keeping of pigs. The herdsman had the obligation to look after the pigs for at least three years, with the obligation to compensate for any damage caused by his responsibility. Half of the offspring belonged to him, which was more favorable than in the case of keeping cows or sheep (Statuta et leges civitatis Buduae, 1988; Katić, 1978; Bujuklić, 1988). The Statute of Skadar talks in more detail about the care of livestock that has been entrusted for safekeeping. First of all, it is stated that shepherds who lead cattle to graze on holidays and Sundays must do this work in such a way that they do not lose their cattle, either their own or someone else's. The livestock owner could hire a maximum of two people during the livestock grazing. One was in charge of guarding the cattle, the other was obliged to bring food. If he hired more than two assistants, he had to pay a fine. The shepherd was obliged to compensate for any damage to the livestock (Statuta di Scutari 2016). Attention is also drawn to the following provision of the Skadar statute, which stipulated that if a shepherd, instead of returning his cattle and those of other villagers, leaves them in the evening and goes to celebrate in the city, he will be responsible for any damage (Statuta di Scutari, 2016). This is about raising livestock, i.e. looking after livestock in such a way that the owners of the livestock hired shepherds who took care of theirs, theirs, but also the livestock of other people who entrusted it to them. The part of the provision where it is said that it applies only to certain days (holidays and Sundays) is particularly significant. This would suggest that a different livestock-keeping regime was in effect on the other days. In the same statute, there is also a provision regulating the loss of a cow during the night in an unknown place. The owner had to be informed about her disappearance before dawn. If the shepherd did not inform him about it, he had to bear the damage himself. The shepherd had to pay for the loss of the cow during the day himself. This provision prescribes

another case of the loss of a cow. If she got lost due to a fly attack, the shepherd had until evening to look for her and inform the owner about it. If he did not find it, he had to compensate the owner of the livestock for the damage (Statuta di Scutari 2016). All the mentioned norms of the Skadar statute on the shepherd's obligations regarding keeping livestock and the measures taken if he does not perform his work conscientiously speak of the efforts of the communal authorities to protect their population from the damage they would suffer due to the loss of livestock. This detailed legal arrangement of this issue concerning the Kotor and Budva communes also speaks of a more pronounced degree of rural life in the Skadar community compared to the other two, which can be seen from the legal norms enacted by its statute. From the above, it can be seen that in Zeta communes there were shepherds who, according to certain norms, took care of livestock, both their own and those of others. It seems that the issue of keeping livestock, shepherds and their obligations is regulated in more detail in the statutes of some Dalmatian communes, e.g. Korcula (Statuta et leges civitatis et insulae Curzullae, 1877) and especially Hvar (Statuta communitatis Lesinae (Phare) 1882-3).

We come to the next question when it comes to the topic of work, which is the use of livestock in Zeta communes. From the available material, it can be seen that cattle (cows, oxen, bison), sheep, goats, horses, donkeys and mules were raised in the mentioned communes. In addition to ordinary cattle, buffaloes were also kept, which were used as draft animals. Cattle were primarily raised for household needs. First of all, it met a significant part of the food needs. Draft and pack animals were used to cultivate the land and transport goods. There was also some benefit from the sale of surplus livestock and their products. It is possible that part of these commodity activities took place through barter for some other agricultural products or other goods necessary for the household. The effort to ensure the supply of livestock products to the population of the commune was of great importance to the commune authorities. When it comes to the commune of Kotor, it is possible that livestock products from the territory of the commune, at least at some point, according to the assessment of its authorities, were not sufficient to meet the needs of that commune, so their export is prohibited. The norm adopted in July 1346 prohibited the purchase of livestock and cheese in the territory of the Kotor commune and their sale outside that city (Statuta Civitatis Cathari, 1616). It is not known whether this measure was conditioned by some other motives (the impossibility of importing these products from some other markets for certain reasons), but it is certainly an example of the communal authorities' concern for the nutrition of their population. Part of the livestock products imported from the Zeta hinterland and the interior were certainly used to meet the needs of the population of the communes, primarily the urban area. Trade in livestock products, as well as other goods, took place in shops and on town squares. (See: Privatni život, 2004).

Certain incomes or services were obtained from renting out a draft and pack animals. Every household at some point did not have the animals necessary

to cultivate the land. The same was true for the transport of goods. Business people who were engaged in trade and transportation of goods were especially interested in this last activity. For these reasons, it was necessary to hire animals for the mentioned activities. We find some information about this special type of obligation relationship in the statutes of Zeta communes. The Kotor Statute contains a norm that more closely regulates the issue of renting cargo animals. Two cases were foreseen when it came to renting these animals. In the first case, when someone would give a pack animal for rent or service, and the owner of the livestock would go with them if the animal died or was stolen, the damage was borne by its owner. If the owner or someone of his does not go, the resulting damage would be borne by the person who hired the animals (Statuta Civitatis Cathari, 1616). The provision on the cows that went to Brskovo and other inland regions also speaks of the rental of pack animals in Kotor (Statuta Civitatis Cathari, 1616; Katić. 1978). Of course, not all the beasts of burden that were used for this activity were the property of people from the district area, but they were mostly the property of Vlach-herdsmen from the hinterland (About this, see: Sindik 1950,). The Budva statute provides somewhat more detailed solutions to the issue of compensation for damage to cargo animals (horses or donkeys) that someone would hire. The main obligation of the person who hired these animals was not to burden them excessively, i.e. more than what was agreed. If a horse or donkey died, the person who hired it was obliged to pay for it. If someone steals those pack animals from him, the damage would be borne by their owner. If he took it at his own risk, he was obliged to pay. If a horse or donkey was stolen in the presence of the owner or his boy, the owner had to pay for the damage, if they were not present, and the person who hired the animals was responsible for the damage. (Statuta et leges civitatis Buduae, 1988; Bujuklić, 1988). The Skadar statute also contains a norm related to the lease of livestock. She talks about hiring oxen to work the land. It was prescribed that if someone hires an ox to cultivate the land of the owner of that animal and his own, permanently or for a certain period, he was obliged to cultivate the land of the owner of the bull fairly as if it were his own. If a fraud of the person from whom the bull was hired or abuse of the animal is determined, if something happens to the bull and because of this the work is not done, if his guilt is proven he is obliged to pay a fine (Statuta di Scutari 2016). How important the bull was for cultivating the land in the same commune is also shown by the provision prohibiting any citizen or foreigner from selling a bull to the slaughterhouse. He could only be sold for fieldwork. The only exception was if it could no longer be used to cultivate the land and only in that case could it be sold (Statuta di Scutari 2016). The hiring of oxen for plowing as well as the plowman's care of them even after the sowing is finished is also recorded in the Korčula statute (Statuta et leges civitatis et insulae Curzullae 1877), while the documents of the Dubrovnik archive offer plenty of data on the leasing of oxen (Blagojević, 2004).

In rural areas such as the Zeta communes, livestock could also be used as a pledge. That type of obligation relations, where the property is specified as a

means of securing the performance of contractual obligations, is regulated in detail in the Kotor Statute (Statuta Civitatis Cathari, 1616). We can assume that under the clause super me et omnia bona mea, by which the debtor committed himself to the creditor to guarantee the fulfillment of contractual obligations in the notarial document, his livestock could also be understood as part of the property (On the pledge in Kotor, see: Bogojević-Gluščević 1999). Unlike the Kotor statute, the Budva statute contains norms that mention livestock as the subject of a pledge. First of all, within the provision on the pledge of movable property, it is briefly stated that an animal cannot be held as a pledge without proof (Statuta et leges civitatis Buduae, 1988; Bujuklić 1988). In the second provision, this issue is regulated in such a way that it is prescribed that no animal may be accepted as a pledge without the surety of a witness, otherwise, the person who would accept as a pledge without a surety had to return the animal to the owner and pay a fine (Statuta et leges civitatis Buduae, 1988; Bujuklić 1988). Therefore, the pledge, in this case, was possible only with the presence of witnesses. As a possible means of pledge, animals are also mentioned in the Skadar statute in the provision related to the payment of the court fee (Statuta di Scutari, 2016). Therefore, in the communes of Zeta, livestock was used as a means of pledge, which was also the case in some Dalmatian communes, e.g. of Hvar (Statuta communitatis Lesinae (Pharae), 1991).

A special type of source that also testifies to livestock farming in the communes is those norms that provide regulations on damage caused by livestock. Those norms are significant in that, in some cases, they can reveal the different attitudes of communal authorities towards sanctioning the same damages, depending on which livestock caused them, which directly speaks of the importance of those animals for the agriculture of the commune. When it comes to the municipality of Kotor, legal norms from the end of the 12th century, related to animal husbandry, have been preserved. The provisions in question were related to the punishment of owners of livestock that caused damage to crops. The first of them is from April 1197 and is located in the Pontifical of Kotor. With this decision, it was stipulated that if someone finds a cow, horse or donkey in his vineyard, he has the right to be compensated for the damage, that is, the effort he invested in cultivating the vineyard. From the owner of that animal, he had the right to take one perper per cow, horse or donkey. As for sheep and other small livestock, it was stipulated that the owner of the vineyard has the right to appropriate them, that is, take them or kill them. The remaining part of the text of this decision was scraped off, but we can assume that it also referred to punishment for damage caused by cattle (Gyug 2016; Gogić 2016). Perper, by the way, was a unit of account that was worth twelve units of local silver money (Ćirković, Mihaljčić 1999). Interestingly, the entire text of this provision (each line) has been crossed out as if it was intended to show that this decision was no longer in force. The following decision on this issue, also recorded in the Pontifical, shows that this was indeed the case. It was passed in April 1203. In its introduction, it is stated that the earlier decision regarding punishment for damage

caused by cattle created numerous problems, which led to numerous court cases. For this reason, the Kotor authorities decided to adopt new measures regarding the sanctioning of damage caused by livestock. The new decision stipulated that if someone found a donkey, horse, cow or another animal on his property, he had the right to appropriate the livestock, and its owner had the obligation to compensate for the damage (Gyug 2016; Gogić 2016).

The statute of Kotor deals with the issue of punishment for damage caused by cattle on arable land. In an undated provision, probably from the 14th century, penalties were prescribed for livestock (horses, mules, donkeys, oxen, cows, sheep, pigs) that caused damage to some arable land (vineyards, orchards, gardens, areas under grain). If a draft animal (horse, mule, donkey, cow) caused damage, its owner was obliged to pay a fine of two perpers to the commune, along with the obligation to compensate for the damage. If another livestock was damaged, the owner who suffered the damage could do whatever he wanted with it, beat or kill it (Statuta Civitatis Cathari, 1616; Gogić 2016). This type of norming changed the norm from 1203, which allowed the appropriation of all livestock that caused damage. Now the owner of the property to which the damage was caused could not freely dispose of the draft stock that caused the damage. Such a measure was probably adopted due to the importance of draft animals in the economy of the population from the area of the commune. From these norms, it can be concluded that draft animals had a slightly different treatment when punished for the damage done.

The aforementioned nondated norm was amended by the aforementioned provision from 1406. The earlier provision that the owner of an ox that caused the damage was to pay a fine of two perpers was changed in such a way that he now pays it to the owner of the property, while for damage caused by mules, horses and donkeys he would still pay the same fine to the municipality. It was also prescribed that the owner of the property (vineyards, gardens, fields) where the damage was caused belongs to two-thirds of the found livestock, one third belonged to the official who found them in damage (Statuta Civitatis Cathari, 1616; Gogić 2016). In this way, the communal authorities actually returned to the norm from 1203. Unlike the one in Kotor, the Budva statute does not decisively specify the types of livestock when prescribing norms for the damage they have caused in the vineyard and the field, making no distinction in punishment for individual types of livestock (Statuta et leges civitatis Buduae, 1988). A similar provision was made in the Skadar statute. It lists cows and horses as livestock for which punishment is prescribed for the damage they have done in the vineyard, but without distinction in their punishment (Statuta di Scutari 2016). In the already mentioned provision of the Scutari statute, which allowed the grazing of horses, cows or donkeys within the sown areas if the cows were tied, a sanction was also provided for in case of damage caused by the cow (Statuta di Scutari 2016). Also, in the statute of Dubrovnik, there is no indication of certain categories of livestock when prescribing the norm for the damage they caused to agricultural crops (Liber statutorum civitatis Ragusii 2002).

When it comes to animal husbandry, not only in the Zeta regions, it should be noted that salt was of great importance for its development and the number of livestock. It was used, first of all, for the preparation and storage of livestock products, but also as a supplement to livestock nutrition. It is known that in the Middle Ages, salt was sold in Kotor and Sv. Srđ, a small square at the mouth of Bojana (Istorija Crne Gore II/1 1970). The proximity of these places where they could get that much-needed item made it easier for the herding population of the Zeta region to engage in animal husbandry.

CONCLUSIONS

In the Middle Ages, animal husbandry represented a significant economic branch that was engaged in by a significant part of the population of Zeta communes. The norms of the statutes of Zeta communes (Kotor, Budva, Skadar) provide data from which a certain picture can be created about animal husbandry in the area of their districts. From the smaller number of statutory norms, one can see the effort of communal authorities to regulate certain issues related to animal husbandry. Significant data on animal husbandry in medieval communes can be obtained from the statutory provisions, the center of which is the regulation of some other issues. The basis for animal husbandry in the communes was the private estates located in its district. In addition to them, pasture lands owned by the communes themselves were used for animal husbandry. From the content of the statutory provisions, it can be seen that the cattle were raised by being kept on their property, within their own household, but also in the way that they were given to grazing by other persons who took care of them. Cattle were raised primarily for the household's needs, food, the sale of surplus agricultural products and their use for cultivating the land and transporting goods. Apart from this, cattle could be used by renting them out and as a means of the pledge.

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EXTRACTION AND SPECTROPHOTOMETRIC QUANTIFICATION OF TOTAL POLYPHENOLIC CONTENT IN ONION

SUMMARY

Consumption of food rich in polyphenols such as fruits and vegetables is of great importance, because it contributes to the prevention of various diseases. Polyphenols are a group of bioactive compounds which can play significant role in preventing various health related problems. One of the richest sources of polyphenols in the human diet is the onion (*Allium cepa* L.). The main objective of the present investigation is quantification of total polyphenolic content (TPC) in the Macedonian local population of onion. A various organic solvents were tested (methanol, ethanol, ethyl acetate and acetone) and their different concentrations (20, 40, 60 and 80%) in order to establish optimum solvent concentration for extraction of polyphenols from onion. Time for extraction was another important parameter which was examined as well. TPC was determined by UV-Vis spectroscopy in accordance with the Folin–Ciocâlteu assay, using gallic acid as a reference standard. The results were expressed as mg gallic acid equivalents GAE/100 g fresh onion. The highest polyphenolic level was determined in 60% methanol (38.81±0.39 mg GAE/100 g), while lower total polyphenolic content was found in the ethyl acetate extracts (27.10±0.35 mg GAE/100 g). Regarding the time for extraction, the highest content of total polyphenols in onion was obtained during extraction of 120 minutes (37.95±0.63 mg GAE/100 g).

Keywords: UV-Vis spectroscopy, onion, extraction, gallic acid, total polyphenolic content (TPC).

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INTRODUCTION

In the recent years there has been great interest in the functional properties of fruits and vegetables, as well as their contribution to human health (Di Lorenzo *et al.* 2021; Chakraborty *et al*. 2022). Fruits and vegetables are source of many useful nutrients, among which polyphenols play an important role (Haminuk *et al.* 2012; Eseberri *et al.* 2022). Polyphenols are organic compounds naturally present in plant foods and characterized by the presence of one or more hydroxyl functional group attached to a single or to multiple aromatic rings (Kondratyuk *et al.* 2004). The relationship between polyphenol intake and human health was investigated with special merit to cardiovascular diseases, hypertension, diabetes, metabolic syndrome, obesity and cancer (Durazzo *et al.* 2019; Cory *et al.* 2018). Among all vegetables, onion (*Allium cepa* L.) is the second major important horticultural crop in the world. It is rich of organic compounds such as polyphenols, flavonoids, anthocyanins and vitamins which have potential beneficial properties for human health (Griffiths *et al.* 2002). The flavonoid compounds, especially quercetin and its derivatives are more common in onion (Slimestad *et al*. 2007; Rodriguez *et al*. 2008). Average total quercetin content in onion (347 mg/kg) is 5 – 10 times higher in comparison with other vegetables and depends on a variety of onion (Lee *et al.* 2014). Taking into consideration that the polyphenols influence on food quality it is essential to study them as major bioactive constituents in onions. Researchers and food processors are increasingly interested in identifying and determining polyphenols in fresh and processed foods (Khoddami *et al.* 2013; Sulaiman *at al.* 2013). The most commonly used methods for determination of total polyphenolic content in food are spectroscopic methods (Bezuneh *et al.* 2015). In the UV/Vis spectrophotometric method colorimetric reactions are widely used, which is easy to perform, rapid and applicable in routine laboratory use, and low-cost (Bueno *et al.* 2012). Folin–Ciocâlteu assay is frequently used for total polyphenolic content evaluation. Namely, polyphenols in plant extracts react with specific redox reagents (Folin–Ciocâlteu reagent) and form a blue complex that can be quantified by visible spectrophotometry (Andressa *et al.* 2013; (Stratil *et al*. 2007; Agbor *et al.,* 2014). Prior quantification of polyphenols first step is selecting a suitable solvent for the extraction of polyphenols from food matrix. Many factors affect the process of extraction of polyphenolic compounds from plant material. The literature data suggested that the recovery, yield and type of polyphenolics in an extracts are influenced among other factors by the type and polarity of extracting solvents and time for extraction (Sulaiman *et al.* 2011; Koffi *et al.* 2010). Selection of the appropriate solvent depends on the food sample among other factors (Lasano *et al.* 2019; Zhou *et al.* 2004).

The main objectives of this study were (i) to evaluate the efficiency of four solvents with different concentration in extracting of polyphenols from fresh onion (ii) to estimate suitable time for extraction and (iii) spectroscopic determination of total polyphenolic content in onion by Folin–Ciocâlteu assay.

MATERIAL AND METHODS

Research material: Macedonian local population of summer yellow onion from village Vogani.

Chemicals and equipment: Folin–Ciocâlteu reagent (Merck), gallic acid (Alkaloid, 99.15%), methanol (Sigma Aldrich, 99.81%), ethanol (Alkaloid, 96%), acetone (Sigma Aldrich), ethyl acetate (Sigma Aldrich, 99.7%), distilled water. All spectrophotometric measurements were made on a Spectrophotometer Varian Cary 50 using a 1.0 cm optical path length glass cell.

Preparation of standard and sample solution: Standard stock solution of gallic acid was prepared when a known amount of the gallic acid was dissolved in methanol (99.81%) in a volumetric flask od 10 cm^3 (Daneshfar *et al.* 2008). Standard test solutions were prepared with dilution from stock solution. All standard solutions were stored at refrigerator at 4 °C. They were stable during the period of analyses. After harvesting the fresh onion samples were stored at room temperature, at a dark place. To prepare the onion samples, fresh onions was skinned, chopped, blended and homogenized.

Extraction of polyphenols from onion: Methanol, ethanol, acetone and ethyl acetate were tested for extraction of polyphenols from onion in different proportions of water (60% and 80%) resulting in different conditions of interactions with the matrices (Nguyen *et al.* 2015). Extraction procedures have been performed by mixing 5 g fresh onion and 25 mL of the desired solvent in a 50 mL conical flask, at room temperature. The conical flask with the mixture was kept under constant stirring using ultrasound bath 15 minutes followed by shaker to 2 hours' time. The onion extracts were filtered through (Whatman No. 1) filter paper and afterwards were stored at refrigerator (4°C) until further analysis.

Determination of total polyphenols: The procedure known from literature was used for quantification of total polyphenols in onion sample with the standard solution of gallic acid. One milliliter (1 mL) of onion's extract was mixed with 5 mL of 1:10 diluted Folin–Ciocâlteu reagent (Singh *et al.* 2017; Kamboj *et al.,* 2015; Agbor *et al*., 2014). The solutions were incubated at room temperature for 5 min. After that 5 mL of 7.5% Na_2CO_3 solution was added and solutions were stored at a dark place 120 minutes in order the reaction to be completed. The absorbance of the reaction mixture was measured at 760 nm. Every determination was made in triplicate. The total phenolic content (TPC) of the extracts was calculated from the regression equation of calibration curve and the results were expressed as mg of gallic acid equivalent (GAE) per 100 g onion.

RESULTS AND DISCUSSION

This study evaluated total polyphenolic content (TPC) after optimization of extraction of polyphenols from onion using different concentration of conventional organic solvents (methanol, ethanol, acetone and ethyl acetate) and different times. TPC in the onion extracts was determined by UV/Vis spectroscopy, applying Folin–Ciocâlteu assay with some modifications (Singh *et al.* 2017; Kamboj *et al.,* 2015; Agbor *et al.,* 2014). This assay is the simplest one

that is available for the measurements of phenolic compounds in different natural products. According to the literature the Folin–Ciocâlteu assay is used for TPC determination in different fruits and vegetables, as it is case in the work of Llupa and coworkers (2022). They used the Folin–Ciocâlteu assay for determination of TPC in quince and sweet cherry (Llupa *et al*., 2022). Colorimetric reactions are widely used in the UV/Vis spectrophotometric method, which is easy to perform, rapid and low cost (Bueno *et al.,* 2012). However colorimetric assay uses a reference substance and measures the total concentration of hydroxyl groups in the plant extracts (Kamboj *et al.,* 2015). The basic mechanism of this reaction is oxidation and reduction, with the phenolic group being oxidized and the metal ion from Folin–Ciocâlteu reagent reduced. In this research for TPC determination as reference standard gallic acid (3,4,5-trihydroxilbenzoic acid) was used (Daneshfar *et al.* 2008). The structural formula and UV spectrum of gallic acid is presented in the Fig. 1.

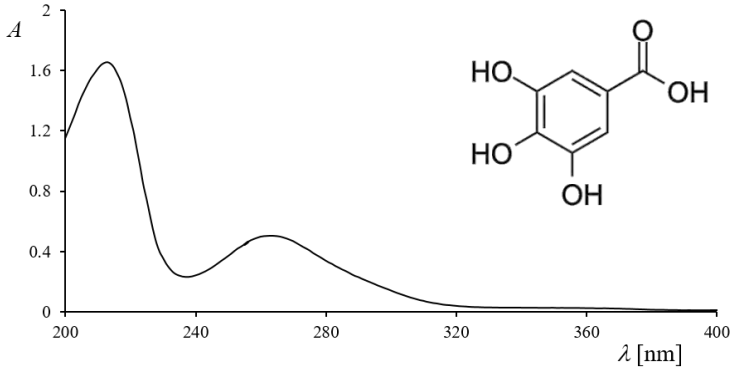

Figure 1. Structural formula and UV spectrum of gallic acid

In the UV spectrum of gallic acid two absorption maxima at around 215 and 265 nm can be observed. As a result of reaction of polyphenolic compounds with Folin–Ciocâlteu reagent blue complex was formed with absorption maximum in the visible region around 760 nm, and it can be quantified by visible spectrophotometry (Kamboj *et al.,* 2015). The intensity of a reaction of phenolic compounds with Folin–Ciocâlteu reagent is proportional to the availability of hydroxyl groups present on the aromatic ring and influences the relative potential of the molecule. Gallic acid has only one ring and three unsubstituted hydroxyl groups and is less influenced by electronic interactions and therefore has higher specific absorptivity and is the best reference substance for determination of TPC (Kamboj *et al.,* 2015).

In this research TPC was determined in the onion extracts obtained with different conventional organic solvents and different times for extraction. The absorbance of the extracts was compared with a gallic acid calibration curve for

estimating the concentration of TPC in the onion sample. The dependence of absorbance on concentration of gallic acid was linear as it is shown in the Fig. 2.

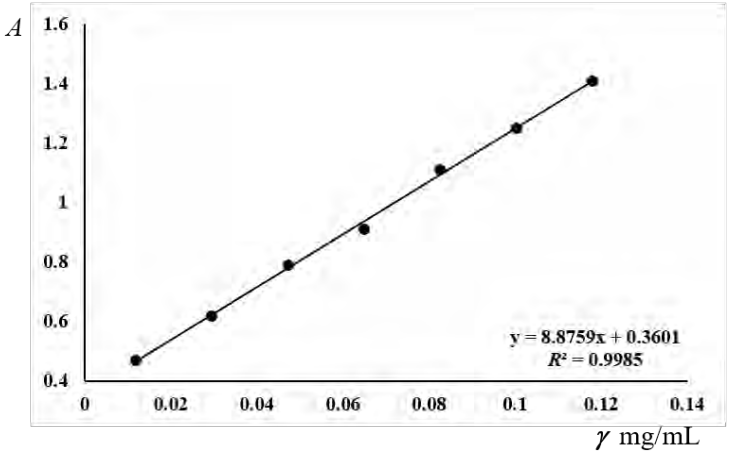

Figure 2. Calibration curve of gallic acid in the concentration range from 0.0118 mg/mL to 0.118 mg/mL

The calibration equation was y=8.8759x+0.3601, with correlation coefficient of 0.9985 which shows excellent linearity over tested concentration interval. TPC was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions, and expressed as mg gallic acid equivalent/100 g fresh weight of onion (mg GAE/100 g).

Extraction of polyphenols from onion with conventional solvents

There are several methods for extraction of polyphenols from fruits and vegetables. One of the most traditional methods uses organic solvents to improve efficiency, quality of extracts, extraction time and the consumption of a solvent. As it is known from literature, selection of solvent has significant effect on the extraction of polyphenols from different sample matrixes (Nguyen *et al.* 2015). Successful extraction of polyphenols from onions influence on the accuracy of the determination. Methanol, ethanol, or their mixture with water as well as ethyl acetate are usually used for the extraction of phenolic compounds (Dai and Mumper, 2010; Ignat *et al.,* 2011). Among organic solvents methanol and ethanol with different water content are the most frequently used, as it is presented in the paper of Krivokapić and coworkers. They determined TPC in two wild-growing plant species (*H. perforatum* L. and *M. officinalis* L) and found the highest values in 80% methanolic, while TPC values in 50% ethanolic extracts were lower (Krivokapić *et al.,* 2021). Furthermore, the Duan *et al.* (2015) found different TPC in onion extracts obtained with methanol and ethanol as solvents (Duan *et al.,* 2016).

The optimization of the extraction conditions in this work was performed by analyzing different organic solvents, and impact of solvent concentration was

investigated as well. The extraction of polyphenols from onion was performed with 80% methanol, 60% methanol, 80% ethanol, 60% ethanol, 80% acetone, 60% acetone, 80% ethyl acetate and 60% ethyl acetate. The average values of TPC expressed as GAE mg/100 g fresh onion ± the confidence interval with a 95% confidence level ($p<0.05$) are given in Table 1. The measurements were carried out in triplicate (n=3), and the standard deviation values are presented in Table 1, as well.

Table 1. TPC in onion extracts obtained using different organic solvents

No.	Solvent	GAE (mg/100 g)	SD (n=3)
1	80% methanol	37.53±0.38	0.33
2	60% methanol	38.81±0.39	0.35
3	80% ethanol	33.68±0.52	0.46
4	60% ethanol	32.38±0.31	0.27
5	80% acetone	37.85±0.60	0.53
6	60% acetone	38.24±0.59	0.52
7	80% ethyl acetate	27.73±0.32	0.28
8	60% ethyl acetate	27.10±0.35	0.31

GAE ± confidence level; SD - standard deviation

According to the results TPC determined in different onion extracts ranged from 27.10±0.31 mg GAE/100 g when ethyl acetate was used as a solvent to 38.81±0.35 mg GAE/100 g when polyphenols were extracted from onion with 60% methanol. As it is presented in Table 1, TPC in onion extracts decreased in following order: 60% methanol >60% acetone >80% methanol >80% acetone >80% ethanol >60% ethanol >80% ethyl acetate >60% ethyl acetate.

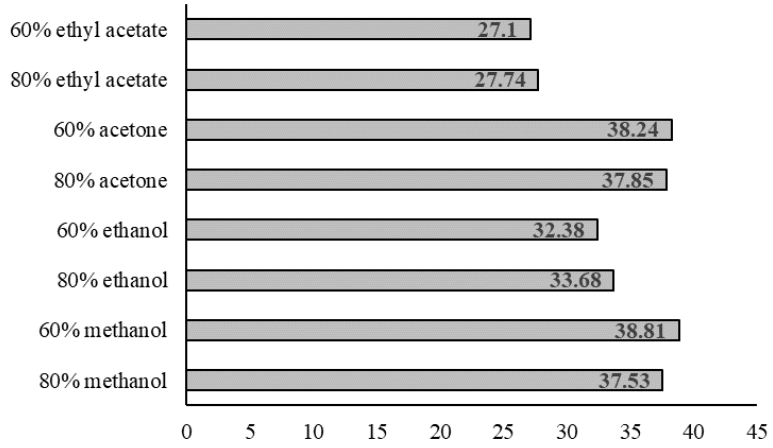

Figure 3. Extraction of polyphenols from onion using organic solvents with different concentration

The obtained results (Table 1, Figure 3) showed that 60% methanol as the extraction solvent gave the best yield of polyphenols compared to the other solvents used in this study. The obtained result is in accordance with the previous studies which reported that using methanol as a solvent for extracting polyphenols in onions is the best option i.e. gives the highest yield of polyphenols in onions' extracts (Singh *et al*. 2017; Hossain *et al.* 2018).

Extraction time

The time required for the extraction plays an important role in efficient extraction of polyphenols from fresh onion and influence on the TPC (Viera *et al*., 2017). Some studies indicated that optimum time for the extraction of polyphenolic compounds fall in the region of 30 to 180 min, but it is dependent on the characteristics and the type of biomass used (Vergara-Salinas *et al.* 2012). In this investigation, the effect of the extraction time on the polyphenolic content in onion extracts was evaluated between 30 min and 180 min, at room temperature. For this purpose, 60% methanol was used as an extraction solvent. TPC obtained during different extraction time expressed as GAE mg/100 g fresh onion and the confidence interval with a 95% confidence level ($p<0.05$) are presented in the Table 2. The measurements were made in triplicate (n=3) and the calculated standard deviation values are given in Table 2, as well.

Table 2. TPC in onion extracts obtained at different extraction times

No.	Time (minutes)	GAE (mg/100 g DW)	SD (n=3)
1	30	36.08±0.32	0.28
2	60	36.37±0.73	0.64
3	120	37.95±0.63	0.56
4	180	36.55±0.54	0.48

GAE ± confidence level; SD - standard deviation

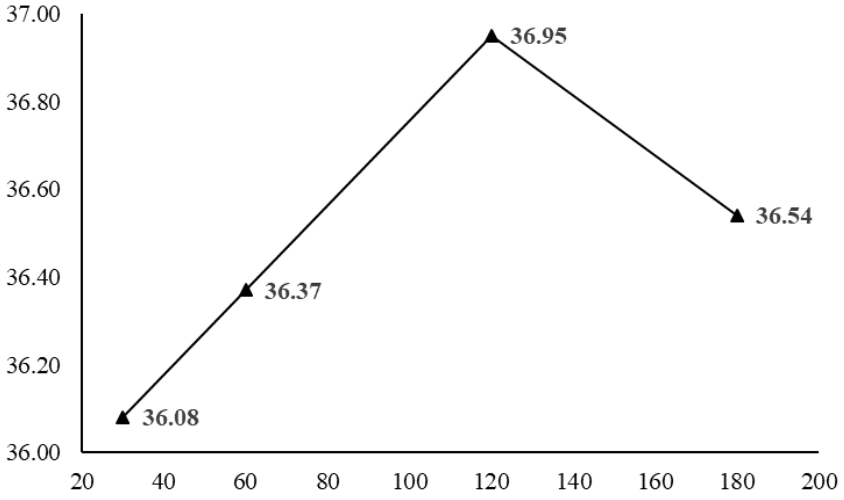

Figure 4. TPC depending on time for extraction

The results in Table 2 show that the TPC values increased gradually with an increase in extraction time from 30 to 120 min, reaching a limit after which they decreased. According to literature when final equilibrium is reached after certain time no more extraction is possible (Durling *et al*. 2007). Higher TPC values were obtained during extraction time of 120 minutes (37.95±0.63) as it can be seen from Table 2 and Figure 4.

The obtained results are in accordance with the previous studies obtained during the testing of extraction time (Pal *et al*. 2019; Souza *et al.* 2009).

CONCLUSIONS

Fruits can be listed as a principal source of polyphenols, among which onion is a great source of polyphenols, especially flavonoids. In the present study TPC was determined by UV-Vis spectroscopy in accordance with Folin–Ciocâlteu assay. This study was conducted in order to investigate which solvent is suitable for extraction of polyphenols from onion and to establish extraction time, as well. Different conventional solvents (methanol, ethanol, acetone and ethyl acetate) in different concentrations (60% and 80%) were evaluated. As it can be concluded from the result different solvent has different extraction efficiency in which the concentration of total polyphenol did not vary significantly with change in solvent. Time for extraction was evaluated, as well. Regarding time for extraction the best results for TPC were obtained during the extraction process of 120 minutes. This study showed that a higher concentration of TPC was found when the time for extraction was 120 minutes. Based on the results presented above, it can be concluded that the most suitable conditions to optimize the polyphenol extraction process from onion is 60% methanol as a solvent and 120 time for extraction.

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EFFECTIVENESS OF SPINOSAD AS A LARVICIDAL AND PUPACIDAL AGAINST CULEX SPECIES IN BENI SUEF GOVERNORATE, EGYPT

SUMMARY

The present study was conducted to evaluate the larvicidal and pupacidal efficacy of Spinosad on *Culex* species in their breeding habitats in village of Saft, Beni Suef Governorate, Egypt. The results showed that Spinosad caused a pronounced toxicity against *C. pipiens*. The total mortality of second and fourth larval instars was achieved by lower concentrations of Spinosad. While the highest pupal morality of *C. pipiens* was obtained by concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions. Spinosad was applied with concentration of 5000 ppm at semi-field experiments and resulted total mortality of larval instars of *C. pipiens*, *C. theileri* and *C. perexiguus* after 24 and 48 h of exposure. The same concentration induced strong pupal mortalities of *C. pipiens*, *C. theileri* and *C. perexiguus* (90, 60 and 70%), respectively after 48 h. The field experiments, Spinosad (5000 ppm) induced total mortality of larval instars of *Culex* species and the pupal mortality was higher in breeding habitat, cement irrigation tank (77.8 %) and decreased to 36.4% in agriculture canal. The present study concluded that Spinosad is a promising larvicide that can be used in treatment of mosquito breeding habitat sites with low efficacy on pupae at some habitat sites such as agricultural canals.

Keywords: *Culex* species; Cement irrigation tank; Breeding habitats; Mosquitos, Agricultural canal

INTRODUCTION

Mosquitoes are a source of nuisance and transmission of diseases to humans and animals. Their risk to transmit diseases through the bite of female mosquitoes increases during their blood meal search before oviposition. The diseases transmitted by female mosquitoes include chikungunya, yellow fever, malaria, filariasis, Zika virus disease and dengue fever. These diseases cause risk to millions

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of people in Egypt and around the world (Southgate, 1979; Meegan *et al*., 1980; Darwish and Hoogstraal, 1981; Warrell, 1993; Elgendy *et al.,* 2018).

Management of mosquito species is applied by using the chemical insecticides (Cetin *et al*. 2006; Aney *et al.* 2018; Eissa *et al*. 2020). However, the frequent use of high application rates of these substances is associated with serious problems, such as increase resistance of insects and detrimental effects on environment, animals and humans (Liu *et al*. 2019; Talipouo *et al*. 2021). Thus, alternative strategies have been examined and used for management of mosquito species, such as Spinosad, essential oils and entomopathogenic fungi (Bond *et al.* 2004; Howard *et al.* 2010; Williams *et al.* 2019; Baz *et al*. 2022).

Spinosad is a bioinsecticide and member of the spinosyn family of insecticides, and one of the most promising alternative methods for controlling mosquito species. It consists of a mixture of two active components, spinosyn A and D derived from a rare, naturally occurring soil dwelling actinomycete bacterium called *Saccharopolyspora spinosa* (Hertlein *et al.* 2010). Several reports were evaluated Spinosad as a larvicidal against mosquito species under laboratory conditions during the last years (Bahgat *et al*. 2007; Perez *et al.* 2007; Benhissen *et al*. 2014; Moselhy *et al*. 2015; Sisodiya *et al*. 2021). However, insufficient knowledge is available on the assessment of Spinosad on mosquito species in their breeding habitats under field experiments (Bond *et al*. 2004; Williams *et al.* 2019). Therefore, the aim of the present study was to determine the efficacy of Spinosad on larvae and pupae of *Culex* species in their field breeding habitats of Beni Suef Governorate, Egypt.

MATERIAL AND METHODS

Insects and bioinsecticide

The field strain of *Culex* species was collected as larvae and pupa from various breeding habitats of tested sites area at village of Saft, Beba, Beni Suef Governorate, Egypt and identified in Research Institute of Medical Entomology, Ministry of Public Health and Population, Dokki, Giza, Egypt. The colony was maintained under the laboratory conditions of 26±2°C and 70±5% R.H. The 2nd and 4th instars larvae and pupae of *Culex* species were collected for the bioassay tests.

Spinosad (Spintor 48% SC) obtained from Dow AgroSciences Co., Cairo, Egypt.

Laboratory experiments

The susceptibility of *C. pipiens* to Spinosad was tested in the laboratory using a methodology adapted from the Elliot larval test (WHO, 1975). Groups of 20 second instar larvae, 20 fourth instar larvae and 20 pupae were separately placed in 150 mL plastic cups containing a solution of Spinosad at one of the following concentrations: (0.125, 0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 ppm) for 2nd and 4th instars larvae and concentrations of Spinosad were increased to 50, 100, 150, 200, 500, 1000, 1250, 2500 and 5000 ppm for pupae. Each concentration was replicated four times. An additional cup contained clean water as an untreated

control. A mixture of ground dried bread and Brewer's yeast pellets (3:1) were added daily larval food. Dead second and fourth instar larvae were recorded after 1 and 24 h while in case of pupae, the pupal mortality was counted after 24, 48, 72 and 168 h.

Semi-field experiments

Semi-field experiments were conducted in village of Saft, Beba distract, Beni Suef Governorate, Egypt. The village of Saft contains several breeding habitats of *Culex* species such as *C. pipiens*, *C. theileri* and *C. perexiguus*. The semi field experiments were performed in plastic tanks (27.0 cm height; 26.5 cm diameter) locality in the mosquitoes breeding habitats sites were filled with 2000 ml water. Then, fifty of larva and pupae were introduced in plastic tank contained water treated with Spinosad (5000 ppm). A small amount (15 mg) of mixture of ground dried bread and Brewer's yeast pellets was provided daily to the larvae. Three plastic tanks were applied for second and fourth instar larvae and pupae of *Culex* species. Dead larvae and pupae were recorded after 1, 24 and 48 h.

Field experiments

The field experiments were performed in two breeding habitats include, cement irrigation tank and agricultural canals in village of Saft, Beba, Beni Suef Governorate, Egypt between March and May 2022. These breeding habitats contained almost *Culex* species mainly *C. pipiens, C. theileri, C. perexiguus* and *C. antennatus*. For field experiments breeding sites were selected which were unlikely to be used for drinking water by animals (Mohamed *et al*. 2022). Spinosad with 5000 ppm were assessed at two breeding habitats. Application doses were achieved by spraying of precalculated amount of oxymatrine with the help of a hand compression sprayer. Prior to spraying, density of immatures was estimated by dipper sampling method using a standard dipper. Density of immatures per dip (five dip x three replicates) was monitored in control and treated habitats at 1 and 24 h. The data obtained at two different times of observation were pooled together to get means of reduction. The percent of reduction in larval and pupal density of *Culex* species was calculated by Mulla *et al.* (1971).

Data analysis

The mortality was corrected according to Abbott's formula (1925) and mortality data transformed by arcine and submitted to a one-way analysis of variance (ANOVA) by using SPSS 21.0 Software (SPSS, Chicago, IL, USA). Tukey's HSD test was used to detect differences between mean mortality values at the 0.05 significance level.

RESULTS AND DISCUSSION

Laboratory experiments

The results demonstrated that concentrations of Spinosad caused higher mortality of larval instars of *C. pipiens* compared to the untreated treatment. The highest larval mortality of second instar (60.0 %) and fourth instar (46.7 %) was achieved by concentration of Spinosad (20 ppm) after 1h of exposure and most concentrations of Spinosad were caused total mortality of two larval instars after 24 h of exposure (Table 1). On the other hand, Spinosad up to 1000 ppm not

affected on treated pupae during 72 h of exposure, then the pupal mortality gradually increased at the higher concentrations and the highest pupal morality of *C. pipiens* was obtained at concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions (Table 2).

Table 1. Mortality percentage of larval instars of *Culex pipiens* after 1 and 24 h after exposure to different concentrations of Spinosad under laboratory conditions

Treatment (Concentratio, ppm)	Mortality percentage of *Culex pipiens* larvae			
	Second larval instar		Fourth larval instar	
	1 h	24 h	1 h	24 h
0.0	0.0g	0.0b	0.0g	0.0c
0.125	3.3fg	100.0a	3.3fg	90.0b
0.25	13.3ef	100.0a	13.3ef	90.0b
0.5	13.3ef	100.0a	16.7de	96.7a
1.0	20.0de	100.0a	13.3ef	100.0a
2.0	26.7cd	100.0a	26.7cd	100.0a
5.0	33.3c	100.0a	33.3bc	100.0a
10.0	46.7b	100.0a	40.0ab	100.0a
15.0	56.7a	100.0a	43.3ab	100.0a
20.0	60.0a	100.0a	46.7a	100.0a

Mean values bearing the same letters within a column are not significantly different ($P<0.05$).

Table 2. Mortality percentage of pupal stage of *Culex pipiens* after 24, 48, 72 and 168 h after exposure to different concentrations of Spinosad under laboratory conditions

Treatment (Concentration, ppm)	Mortality percentage of *Culex pipiens* pupae after (h)			
	24	48	72	168
0.0	0.0d	0.0c	0.0d	0.0e
50.0	0.0d	0.0c	0.0d	0.0e
100.0	0.0d	0.0c	0.0d	0.0e
150.0	0.0d	0.0c	0.0d	20.0d
200.0	0.0d	0.0c	0.0d	30.0c
500.0	0.0d	0.0c	0.0d	33.3c
1000.0	0.0d	0.0c	0.0d	33.3c
1250.0	60.0c	73.3b	73.3c	73.3b
2500.0	80.0b	86.6a	86.6b	86.6a
5000.0	86.6a	90.0a	93.3a	93.3a

Mean values bearing the same letters within a column are not significantly different ($P<0.05$).

Semi-field experiments

The data presented in Table (3) indicated that Spinosad (5000 ppm) caused total mortality of larval instars of three *Culex* species (*C. pipiens, C. theileri* and *C. perexiguus*) in treated plastic tanks compared to the control treatment after 24 and 48 h. While the highest pupal mortality percentages of three *Culex* species (*C. pipiens, C. theileri* and *C. perexiguus*) were achieved after 48 h of exposure to 5000 ppm of Spinosad (90, 60 and 70%), respectively.

Table 3. Mortality percentages in immature stages of *Culex* species after 1, 24 and 48 h exposures to 5000 ppm of Spinosad in plastic tank under semi-field conditions

Concentration, ppm	Mortality percentage of								
	Second larval instar			Fourth larval instar			Pupal stage		
	Cx. pipiens	*Cx. theileri*	*Cx. perexiguus*	*Cx. pipiens*	*Cx. theileri*	*Cx. Perexiguus*	*Cx. Pipiens*	*Cx. theileri*	*Cx. perexiguus*
0.0	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
5000.0 after (1 h)	40.0 b	50.0 b	40.0 b	30.0 b	40.0 b	40.0 b	0.0 b	0.0 b	0.0 b
5000.0 after (24 h)	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	90.0 a	60.0 a	70.0 a
5000.0 after (48 h)	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	90.0 a	60.0 a	70.0 a

Mean values bearing the same letters within a column are not significantly different ($P<0.05$).

Field experiments

The percent of reduction in density per five dip of larval instars of *Culex species, C. pipiens, C. thelerei, C. perexiguus* and *C. antennatus* in treated cement irrigation tanks and agriculture canals with Spinosad is presented in Tables (4 and 5). The results were demonstrated the complete reduction (100%) of larval density of *Culex* species were achieved at 5000 ppm of Spinosad at both breeding habitats, cement irrigation tank and agriculture canal after 24 h.

Table 4. Percent of reduction in density of *Culex* species larvae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in cement irrigation tank under field conditions

Concentration, ppm	Percent of reduction of second larval instar							
	C. pipiens		*C. theileri*		*C. Perexiguus*		*C. antennatus*	
	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)
0.0	5.0	0.0 c	2.0	0.0 c	1.0	0.0	3.0	0.0
5000.0 after (1 h)	3.0	40.0 b	1.0	50.0 b	1.0	0.0	0.0	100.0
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0	0.0	100.0
Percent of reduction of fourth larval instar								
0.0	8.0	0.0 c	5.0	0.0 c	3.0	0.0 c	3.0	0.0 c
5000.0 after (1 h)	6.0	25.0 b	3.0	40.0 b	1.0	66.7 b	2.0	33.3 b
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a

Mean values with the same letter within a column are not significantly different ($P<0.05$), PR% = Percent of reduction.

Table 5. Percent of reduction in density of *Culex* species larvae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in agriculture canal under field conditions

Concentration, ppm	Percent of reduction of second larval instar							
	Cx. pipiens		*Cx. Theileri*		*Cx. perexiguus*		*Cx. Antennatus*	
	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)
0.0	6.0	0.0 c	6.0	0.0 c	1.0	0.0	1.0	0.0
5000.0 after (1 h)	4.0	33.3 b	3.0	50.0 b	0.0	100.0	0.0	100.0
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0	0.0	100.0
Percent of reduction of fourth larval instar								
0.0	11.0	0.0 c	7.0	0.0 c	4.0	0.0 c	3.0	0.0 c
5000.0 after (1 h)	8.0	27.3 b	6.0	14.3 b	2.0	50.0 b	2.0	33.3 b
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a

Mean values with the same letter within a column are not significantly different ($P<0.05$), PR% = Percent of reduction.

On the other hand, the percent of reduction in pupal density of *Culex* species in two breeding habitats was decreased to 77.8 and 36.4% in cement irrigation tank and agriculture canals, respectively after 24 h (Table 6).

Table 6. Percent of reduction in density of *Culex* species pupae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in two breeding habitats (cement tank and agriculture canal) under field conditions

Concentration, ppm	Percent of reduction in density of pupae			
	Cement tank		Agriculture canal	
	No. density	PR (%)	No. density	PR (%)
0.0	9.0	0.0 c	11.0	0.0 c
5000.0 after (1 h)	7.0	22.2 b	8.0	27.8 b
5000.0 after (24 h)	2.0	77.8 a	7.0	36.4 a

Mean values with the same letter within a column are not significantly different ($P<0.05$), PR% = Percent of reduction.

Efficacy of Spinosad on mosquito species has been reported by several studies (Bond *et al*. 2004; Williams *et al*. 2019; El Sayed *et al.* 2020). These previous reports indicated that Spinosad had the ability to manage mosquito larvae as biocide. Our results indicated that Spinosad caused higher toxicity against *C. pipiens* and the total mortality of second and fourth larval instars was achieved at lower concentrations. While the highest pupal morality of *C. pipiens* was obtained at concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions. These findings were matched with previous studies demonstrated efficacy of Spinosad on *Culex* species such as Bahgat *et al.* (2007) showed that Spinosad was tested in laboratory and field against some *Culex* species in El-Ismailia Governorate and found the LC_{50} value of the liquid formula of Spinosad for *C. pipiens* was 0.002 ppm after 24 h. This higher toxicity of Spinosad was observed on fourth instar larvae of *C. pipiens* (Benhissen *et al.* 2014). Moselhy *et al*. (2015)

revealed that Spinosad was the most effecting tested insecticide on *C. pipiens* larvae. El Sayed *et al.* (2020) showed that efficacy of Spinosad was studied to control *C. pipiens* larvae by different concentrations. The mortality percentage of larvae was increased by increasing concentrations and time. While 100% mortality was obtained at the concentration 1000 µl/10 ml of Spinosad after 48 h. This higher insecticidal efficacy of Spinosad on *Culex* species may be due to their mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptors (Salgado, 1998; Watson, 2001).

The results of current study revealed that Spinosad (5000 ppm) showed pronounced toxicity against larval stages of *Culex* species with total mortality of larval instars, while the pupal mortality was more than 60% in semi-field experiments. Similar results were obtained by Bond *et al.* (2004) found that Spinosad 1 ppm compared with the standard temephos (Abate1) 1% granules 100 g/m3 water prevented *Aedes aegypti* breeding in plastic containers of water for 8 weeks, at 10 ppm Spinosad prevented breeding for > 22 weeks. Gharib (2021) found that Spinosad showed the highest larvicidal effect against *C. pipiens* with mortality percentage (69.5%) in semi field experiments. The same finding was obtained previously by other insecticides on mosquito species (Thavara *et al.* 2004; Marcombe *et al.* 2011; Mohamed *et al*. 2022).

The results of current study demonstrated that Spinosad (5000 ppm) caused total mortality of larval instars of *Culex* species and the pupal mortality was higher in breeding habitat, cement irrigation tank (77.8 %) and decreased to 36.4% in agriculture canal. Similar results were obtained by Bond *et al*. (2004) showed that the field experiment of Spinosad (5 ppm) caused completely eliminated reproduction of *A. aegypti* for 13 weeks. They also predict that Spinosad is likely to be an effective larvicide for treatment of mosquito breeding sites. Williams *et al.* (2019) examined the efficacy of Spinosad for the control of mosquito larvae present in experimental tires in Veracruz State, Mexico in the period 2015–2016 and found that Spinosad granules provided control of larvae and pupae of *A. aegypti*, *A. albopictus* and *Culex* spp. in used tires in Veracruz State, Mexico.

CONCLUSIONS

It could be concluded that Spinosad is highly effective for the management of mosquitos. It's able to achieve total larval mortality of *Culex* species in their field breeding habitats such as cement irrigation tank and agricultural canal as strong larvicide alternative to synthetic insecticides. The results demonstrated also, the low efficiency of Spinosad on pupal stage of *Culex* species in field experiments. Further studies should be done to assess Spinosad effectiveness as pupalcide against mosquito species in various ecological regions, different mosquito populations, and species in their field breeding habitats.

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DETERMINATION OF RAW MILK QUALITY BY TOTAL BACTERIAL COUNT AND SOMATIC CELL COUNT IN DIFFERENT REGIONS IN REPUBLIC OF KOSOVO

SUMMARY

The aim of this research was to determine the quality of raw milk by total bacterial count (TB) and somatic cell count (SCC) in the Republic of Kosovo. This research was carried out in a dairy plan in the territory of the Republic of Kosovo. The raw milk processed in the dairy was used for analysis during the research period. Raw milk samples were taken by every producer who delivered milk at the dairy for a period of four months (January to April). Raw milk samples came from five different areas of the territory of the Republic of Kosovo. In total 90 raw milk samples were analyzed. During the research it is determined that the quality of raw milk deviates in terms of the regulations requirements for milk quality. The average number of somatic cell counts was 657,055 cells/ml and the total bacterial count was 2,626,616 CFU/ml of the total number of the analyzed samples. There were not statistically significant differences of the raw milk of the five examined regions.

Keyword: raw milk, Kosovo, somatic cells, total bacterial count

INTRODUCTION

Milk must be specific to human consumption i.e. come from well-nourished healthy lactating animals. This means that the milk of infected animals (resulting from inflammation of the udder), undergoing veterinary treatment is excluded. Milk must be maintained at a temperature of 4 °C or below during all operations (Kohler, 2013), and because it is a perishable product transporters must ensure proper handling and hygiene (Guetouache *et al.,* 2014). Milk is sterile during secretion in the alveoli of the udder. Its contamination with

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microorganisms starts during and after milking (Tamime, 2009). The first contamination of milk with bacteria occurs in the teat canal. Just passing through the channel, the milk is contaminated with 100 – 1,000 CFU (Colony Forming Units)/ml. Microorganisms in raw milk originate from different types of contamination, such as: the udder of the cows, the surface of the cows, from the air as well as from the surfaces of the equipment with which the milk is in contact during milking, storage and transport (Bytyqi *et al.,* 2011). Contamination can be with thermophilic or mesophilic microorganisms. Milk is an excellent environment for the development of such microorganisms, especially mesophilic ones, whose reproduction depends on temperature. So their number can double every 15 minutes at a temperature of 30-37°C (Alcázar, 2011). Milk is a good medium for the growth of many microorganisms due to its high water activity, near-neutral pH, and available nutrients (Frank, 2007).

The microbiological quality of raw milk can be affected by several factors, such as milking, type of farm (conventional, organic) and season of the year (Bogdanovicova K, *et al.,* 2016). Other factors that have an influence on the microbiological quality are the type of nutrition of the animals, the stage of lactation as well as the way of keeping the animal, i.e. the environment in which they live (Dunge, 2016). The type of microorganisms present in raw milk depends on the temperature and time of storage, as well as the procedure with the milk during and after milking (Tamime, 2009).

The total number of somatic cells count (SCC) in milk is a significant indicator of the health of the mammary udder. In addition, somatic cells are a good diagnostic tool that allows early detection of the subclinical or acute form of mastitis (Green *et al*., 2004). That is why they represent a significant component in the monitoring program at the farm level (Schukken *et al.,* 2003). The number of somatic cells in the milk is an indicator of the health status of the mammary gland in cows, an indicator of the stability of the milk and its quality for consumption, as well as an indicator of the losses that producers have during mastitis (Brahma *et al*., 2017). A high number of SCC reduces the durability of milk, and negatively affects the sensory characteristics of the final products (Bytyqi *et al.,* 2011). Also, SCC are correlated with subclinical mastitis and changes in physicochemical properties in raw milk (decreased milk quantity, changes to milk consistency (density), decreased the percentage of protein). Additionally, a higher number of SCC can lead to worsening the milk hygiene and even contain pathogenic microorganisms (Toepel., 2004).

Raw milk should be analyzed in order to identify potential deviations of physicochemical properties and microbiological contamination. Microbiological contamination during milking and after milking is a huge problem in the dairy industry. Some microorganisms can reduce the quality of the milk and can be responsible for milk spoilage, and affect the shelf life of raw milk (Kadriu, 2021).

The first step for controlling the quality of raw milk should be done on the farm level. Farmers are the ones who need to improve the conditions of keeping, feeding and milking the cows in order to obtain quality and safe raw material for

processing (Tamime, 2009). Additional control should be followed by the dairy industry as well as by government institutions in order to improve the current situation in the Republic of Kosovo (Kadriu, 2021).

MATERIAL AND METHODS

This research was carried out in a dairy plan in the territory of the Republic of Kosovo. The raw milk processed in the dairy was used for analysis during the research period. Raw milk samples were taken by every producer who delivered milk at the dairy for a period of four months (January to April). Raw milk samples came from different areas of the territory of the Republic of Kosovo (region 1 - Mitrovica, region 2 - Pristina, region 3 - Gnjilane, region 4 - Pec, and region 5 - Prizren). In total 90 raw milk samples were analyzed 27 samples were analyzed from region 1, i.e. 30% of the total number of samples, 13 samples (14.44%) were analyzed from region 2, 21 samples (23.33%) were analyzed from region 3, and 21 samples (23.33%) were analyzed from region 4.19 samples (21.11%) and 10 samples were analyzed from region 5, i.e. 11.11% of the total number of analyzed samples.

The total number of microorganisms was examined using the BactoScan ™ FC - which counted the bacterial colonies in the milk (CFU). The instrument works on the principle of fluorescence flow cytometry according to the ISO 21187 standard. The number of somatic cells was examined using the Fossomatic ™ Minor. The instrument works on the principle of the fluoro-opto-electronic method according to the standard ISO 13366-2.

The obtained results were compared with official quality regulations for raw milk in Kosovo (Table 1) (MAFRD., 2006).

Table 1. Milk quality standard in Kosovo (CFU/mL and SCC/mL in milk).

Standard for fresh milk	Extra Class	I Class	II Class	III Class
CFU/Ml	<80,000	<100,000	<200,000	<500,000
SCC/Ml	<300,000	<400,000	<500,000	<600,000

Additionally, the number of somatic cell counts was categorized into four categories in order to see how many samples meet the regulations criteria.

-Category I (≤ 200,000 cells/ml)
- Category II (from 200,001 – 400,000 cells/ml)
- Category III (from 400,001 – 600,000 cells/ml)
- Category IV (≥ 600,001 cells/ml)

RESULTS AND DISCUSSION

From the data presented in Table 2, we can determine that the average number of the somatic cell count, in the examined samples, varies in the range from 414,383 SCC/ml (region 2) to 783,052 SCC/ml (region 4), with an average

value of 657,055 SCC/ml. In all five regions the average number of SCC, do not meet the requirements according to the Rulebook for determining the specific requirements of hygiene of food of animal origin (Official Gazette R. Kosovo 12/2011). Similar results were presented by the Food and Veterinary Agency of Kosovo where for 2007 year, 57.31% of samples had lower than or equal to 400,000 SCC/ml and on the other hand 29.44% of the samples have more than 600,000 SCC /ml (Musliu *et al*., 2009).

Table 2: Changes of SCC/ml in raw milk according to the studied regions

Parameter	Region	N	$\underline{x}$	SD	Min	Max
SCC/ml	1	27	713,777	370,521	78,000	1,499,000
	2	13	414,384	436,251	77,000	1,616,000
	3	21	581,238	1,587,920	8,000	7,443,000
	4	19	783,052	913,432	192,000	2,802,000
	5	10	739,200	726,330	78,000	2,691,000
	Total	90	657,055	932,507	8,000	7,443,000

Even more, when we look at the variations of the minimum and maximum values of the number of somatic cells, which range from 8,000 SCC/ml to 7,443,000 SCC/ml, respectively, they point to the fact that there are huge differences between regions, but also in the regions themselves. Even if the average number of SCC in the raw milk is 370,521 SCC/ml (region 1) the minimum and maximum number of somatic cells ranges from 78,000 SCC/ml to 1,499,000 SCC/ml.

According to the obtained data on the number of somatic cells, there are also data on the number of CFU/ml, where it is clearly seen that the average number of CFU/ml for all regions in the examined period was 2,616,616 CFU/ml (table 3). Variations of the same parameter ranged from 1,365,071 CFU/ml (region 4) to 3,803,000 CFU/ml (region 2). Such results are not in accordance with the Rulebook establishing the specific requirements of hygiene of food of animal origin (Official Gazette R.Kosovo 12/2011) for the hygienic quality of the processed milk. These data indicate poor hygiene during milking, also improper milk storage, as well as a higher prevalence of subclinical mastitis.

Table 3: Changes of CFU/ml in raw milk according to the studied regions

Parameter	Region	N	$\underline{x}$	SD	Min	Max
CFU/ml	1	25	3,363,520	9,022,032	70,000	45,742,000
	2	12	3,803,000	4,164,898	124,000	14,791,000
	3	14	2,144,642	1,946,187	126,000	5,674,000
	4	14	1,365,071	2,117,198	85,000	5,299,000
	5	8	1,519,125	1,727,863	90,000	4,574,000
	Total	73	2,616,616	5,698,770	70,000	45,742,000

In general, if we consider the quality of milk obtained in the territory of the Republic of Kosovo during a period of four months, it can be noted that in terms of hygienic quality, the obtained results exceed the requirements according to the Regulation (Standards for quality and categorization of raw milk, 20/2006). According to Bytyqi *et al.,* (2014) about 29.6% of the analyzed milk samples belong to extra class milk (SCC/mL<300.000), followed by milk quality class III-rd (24.3%), I-st (8.5%) and II–nd (8,2%), where the SCC/mL are ranged from >300.000 till <600.000 for the respective year. On contrary of the total number of analyzed bulk milk samples, 29.5% samples do not fulfill the milk quality standards, poor quality (SCC/mL >600.000). Additionally, Uka E *et al,.* (2018) analyzed 11,482 milk samples, in 2017 where 35% from the samples belong to extra class milk (CFU<80,000) and 46% to III-rd class (CFU<500,000). Similar results where presented in 2018, where 33% from the analyzed 8,795 milk samples were classified as extra milk class, and 44% to III-rd class. Different results were presented by Bytyqi *et al.,* (2010) where in their research for 2008 and 2009, the requirements for extra milk (in terms of SCC/ml) meet 78.45%, and 70.88%, from farms, respectively. They indicate that there are significant differences between the examined 13 farms in the territory of the Republic of Kosovo in terms of the hygienic quality of the milk. According to the results presented by Visciano & Schirone (2022) who analyzed milk samples in Italy (Pescara and Teramo) only 23 (9.6%) out of 240 samples exceeded the regulatory limit for SCC, and the maximum found value was 2,351,000 cells/mL.

On the other hand, Bytyqi *et al.,* (2011) in their research analyzed 70% of raw milk processed in 14 largest dairies in Kosovo. According to their results for 2008 only 20% of the milk producers in Kosovo meet the requirements and around 10% meet the standards given in the Regulations for the following calendar year 2009 (CFU<100,000 CFU/ml). Regarding the total somatic cell count, 80% of the raw milk samples met the 2008 Regulations in Kosovo (SCC<600,000 cells/ml) and 66% met the 2009 Regulations (SCC<400,000 cells /ml).

From the total number of analyzed samples for (N=90), 20 samples, i.e. 22.22% refer to samples where the number of somatic cells is up to 200,000 cells/ml, while 21 samples refer to the number of somatic cells from 200,001 to 400,000 cells/ml, i.e. 23.33% (table 4) or about 45% of the tested samples meet the standards for hygienic quality of the milk. Similar results were presented by Bytyqi *et al*., (2011) and Bytyqi *et al.,* (2010). Statistically significant results were obtained between the fourth category with the other three categories ($p<0.01$). However, these data, as well as those from previous studies, point to the fact that almost half of the dairy animals do not show signs of inflammation of the udder, but as an additional problem are the poor hygienic conditions during milking, as well as the inadequate storage and transportation of the raw milk to the dairy. Additionally, this is compounded by the fact that the transportation of raw milk to the dairy is not standardized. In addition, not only the means of transport are under different ownership, the transport also differs, that is, a total

of six different types of milk transport are defined in the territory of the Republic of Kosovo. For each transport system, it is specifically defined where and when the milk sample should be taken (Bytyqi *et al.,* 2011).

Table 4: Changes in the hygienic quality of raw milk shown by categories according to the number of somatic cell count

Parameter	Category of SCC	N	$\bar{x}$	SD	Min	Max
SCC/ml	I	20	91,800[a]	58,250	8,000	192,000
	II	21	289,428[a]	67,284	201,000	377,000
	III	17	470,647[a]	34,660	415,000	519,000
	IV	32	1,350,625[b]	1,295,216	623,000	7,443,000
	Total	90	657,055	932,507	8,000	7,443,000

*Differences of values with different superscripts in the same group are statistically significant at the level: a:b $p<0.05$

Table 5 shows the total number of microorganisms, by category, according to the number of somatic cells. The obtained results point to interesting data where it can be seen that in the first category (≤200,000 SCC/ml) we have an extremely high total number of microorganisms 1,978,333 CFU/ml, which indicates the poor hygienic conditions in which raw milk is obtained and stored. The total number of microorganisms also increases in the other two categories (the second and the third). In addition, the raw milk that had the highest somatic cell count (≥600,000 SCC/ml) also had the highest total bacterial count of 3,151,290 CFU/ml. In addition to the fact that there are variations in this parameter, no significant differences were observed between the examined groups ($p>0.05$).

Table 5: Changes in CFU/ml in raw milk shown by category according to somatic cell count

Parameter	Category according to SCC	N	$\bar{x}$	SD	Min	Max
CFU/ml	I	12	1,978,333	2,603,418	124,000	8,671,000
	II	18	2,407,944	3,695,364	70,000	14,791,000
	III	12	2,186,666	1,064,303	545,000	3,489,000
	IV	31	3,151,290	8,169,771	78,000	45,742,000
	Total	73	2,616,616	5,698,770	70,000	45,742,000

CONCLUSIONS

The dairy industry needs quality raw milk in order to produce quality dairy products. A large number of factors can influence the quality of milk, among which, the most important component, naturally present, is the number of somatic

cells. Due to these reasons the number of somatic cells is used as an indicator of the health condition of the udder and the quality of milk. Furthermore, the increase of the total number of microorganisms can be one additional factor for worsening the hygienic quality of milk.

The quality of the raw milk in Kosovo still is very poor and must be improved. Quality milk production is the ultimate goal and goal of any milk producer. The low quality of milk has a negative impact on all segments of the dairy industry, especially in the fresh milk processing segment. Milk quality is the primary factor and ultimate goal of any milk producers in order to obtain quality milk products. Increased SCC is associated with milk reduction and additionally shorter shelf life of the produced milk products. Only when the primary raw material has good quality, quality milk products will be obtained by milk producers.

During the research it is determined that the quality of raw milk deviates in terms of the regulations requirements for milk quality. The average number of somatic cell count was 657,055 cells/ml and the total number of microorganisms was 2,626,616 CFU/ml of the total number of the analyzed samples (N=90). There were not statistically significant differences of the raw milk of the five examined regions.

Current milk quality standards applied in Kosovo do not meet the EU criteria, therefore, Kosovo farmers should be aware that in the very near future they have to meet the EU milk quality standards in order to remain and compete on the market.

The results obtained in this research will make significant contributions to the scientific community, while they also can have applicative character. In this way, the producers of dairy products and the dairy industry can see the possibilities for mutual cooperation in order to improve the quality of the primary raw material.

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DIVERSITY OF FISH FAUNA OF THE ZETA RIVER (MONTENEGRO) AND ABSENCE OF TROUT SPECIES CAUSED BY INADEQUATE MANAGEMENT

SUMMARY

A comprehensive study of fish diversity, relative and absolute biomass of registered species in Zeta River, Montenegro was accomplished. The data were collected during 2019 from three major ecological sectors of the river. A total of 14 fish species were recorded. The highest relative and absolute biomass were recorded on the Lower Zeta – Bjelopavlići – the lower part of the watercourse.

Data presented in this paper shows a dramatic decline in both, the abundance and biomass of trout species from this river. Although in the 2019th river Zeta watercourse in Bjelopavlići Valley was proclaimed as a Park of Nature, we haven't detected any benefits for the fish species, especially for the salmonid ones and their abundance continued with decreasing trend. Most probably the bad practice of poaching with forbidden tools just continued no matter on newly proclaimed protection status. It is shown that proclaiming some area or river in the protected area of any level doesn't benefit to biodiversity if it is not followed with precise management plans and strict implementation.

Keywords: Ichthyofauna, CPUE, NPUE, absolute biomass, management

INTRODUCTION

The freshwater fish fauna of Montenegro currently consists of 89 species (Marić, 2019). The ichthyofauna of the inland waters of Montenegro can be divided into two groups: the Danube (Black Sea) and the Adriatic Sea. The Zeta, the Morača and the Bojana Rivers together with the Skadar Lake form the main part of the Adriatic River Basin, flowing into the Adriatic Sea. The River Zeta is the largest tributary of the Morača. In terms of biodiversity, the Zeta river is one of the most important rivers in the Adriatic basin of Montenegro. The autochthonous ichthyofauna of the River Zeta is represented by 20 species. Two of them, i.e., *Salmo zetensis* (soft-muzzled trout) and *Barbatula zetensis* (Zeta

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stone loach), are endemic to Montenegro (Marić, 2019). Cyprinid fish that form half of the autochthonous fauna dominate in the lower part of the river, while the upper part is populated by *Salmo zetensis, S. marmoratus, S. farioides*, and some cyprinids (Marić, 2018).

In terms of both biodiversity and fishery, Lake Skadar is the richest and most important area in Montenegro. The largest number of published research papers from the area of Montenegro is precisely related to the area of Lake Skadar and its fish resources (Drecun and Ristić, 1964; Ivanović *et al*., 1983; Janković, 1975; Ivanović, 1973; Milošević *et al*., 2011; Milošević and Marić, 2012; Milošević and Mrdak, 2016; Mrdak *et al*, 2017; Mrdak *et al*., 2017 b; Marić, 2018). Until now very few researches were conducted on fish biodiversity status of the Zeta River. Data on fish diversity were most often presented in the form of a list of species as well as the fishing potential of certain areas of the River Zeta (Drecun, 1951; 1981). The subject of more detailed research was only the endemic soft-mouthed trout. Results of genetic investigation on twelve microsatellite loci as well as lack of hybridization with the putative brown trout unequivocally confirm that the soft-muzzled trout from the Zeta River is a species distinct from the putative brown (*Salmo cf. farioides*) and marble (*Salmo marmoratus*) trout that live in sympatry with it (Mrdak *et al.,* 2012).

The Zeta River is categorized as a river of national importance. It has been declared a Park of Nature in 2019, by the decision of the Government of Montenegro, at the initiative of the Municipality of Danilovgrad. Thus, Zeta became an area of special importance for Montenegro, having in mind the significance of its biodiversity.

The whole lower part which runs through the Bjelopavlići Valley is under protection as a Park of Nature, while the upper part of Nikšić Valley is not protected and it was under significant human impact due to building of the hydro system for the need of the "Perućica" hydro energy plant. Bearing in mind the above as well as the fact that until now there has been no systematic research on fisheries resources of the Zeta River, this study was designed to provide answers to the following questions:

1. Which species are most abundant in the study area?
2. What's the situation in terms of relative and absolute biomass three study parts of the river?
3. What are the main reasons for fish biodiversity depletion in the Zeta River with recommendations?

MATERIAL AND METHODS

Study area

The total length of the Zeta River is about 85 km with a drainage basin area of 1,597 km^2. The river originates in Gornje Polje from the rivers Sušica and Rastovac. From the springs of Perućica and Oboštica, the watercourse of Lower Zeta is formed, which flows into the river Morača. Until its conflow with Morača

river, the lower Zeta receives several tributaries, the most important of which is Sušica with a length of 15 km. (Radojičić, 2005).

Sampling

Research on the Zeta River was conducted during the 2019. Investigating area was divided into three parts:

1. Upper Zeta-Nikšić valley (localities: Brezovik, Duklov most);

2. Lower Zeta-Bjelopavlići – the upper part of the watercourse (localities: Glava Zete, Ostrvo, Tunjevo);

3. Lower Zeta – Bjelopavlići – the lower part of the watercourse (localities: Danilovgrad, Pričelje, Vranjske njive).

Fish were sampled by electrofishing and commercial or standard benthic MMG nets (European standard EN 14757 - European Committee for Standardization 2015). A standard electrofishing gear (SUSAN-735MP) was employed according to technical instructions. All species were determined to species level. Individual MMG were 30 m long and 1.5 m high, resulting in a net surface area of 45 m^2. Each net consisted of twelve 1.5×2.5 m panels of different mesh sizes in the following sequence: 43 mm, 19.5 mm, 6.25 mm, 10 mm, 55 mm, 8 mm, 12.5 mm, 24 mm, 15.5 mm, 5 mm, 35 mm and 29 mm (knot to knot). Each fish caught was identified to a species level. Total length (TL, to the nearest mm) and total weight (TW, to the nearest g) of each specimen were measured separately. Catch per unit of effort (CPUE) and number per unit of effort (NPUE) were calculated for all sampled species as a relative measure of species biomass and species abundance, respectively.

CPUE and NPUE were calculated for individual species as follows:

− CPUE (g/m2) = biomass of total catch (in grams) divided by surface of electrofishing transect or with total net surface.

− NPUE (individuals/m2) = total number of individuals per MMG (all panels) divided by surface of electrofishing transect or with total net surface.

For calculating absolute biomass, the mean value of total biomass of all transects in the appropriate river sector was extrapolated on one hectare surface (electrofishing sampling). In case of net sampling, we calculate that catch in one net is equal to the total biomass of the surface of a square with the diagonal length of that net. The mean value of calculated biomass of each such square from appropriate river sector was extrapolated on one hectare surface.

RESULTS AND DISCUSSION

This paper provides the main information and identifies the knowledge gaps about the fish resources in the Zeta River which is an important identifying factor in planning the next steps towards conservation of fish fauna and sustainable fishing in the country.

1.Upper Zeta – Nikšić valley

a)Fish assemblage

Three species have been registered in the area of upper Zeta – Nikšić valley (Table 1). During the survey period, the highest relative abundance was recorded for *Phoxinus phoxinus* (Table 2).

Table 1. Registered species, No-number of individuals; %-percentage in the sample by locality

UPPER ZETA – NIKŠIĆKO POLJE			
Locality	**Species**	**No**	**%**
Brezovik	*Salmo labrax*	7	5 %
	Phoxinus phoxinus	121	86.5 %
	Squalius cephalus	12	8.5 %
Duklov most	*Salmo labrax*	5	2.5 %
	Phoxinus phoxinus	174	82 %
	Squalius cephalus	33	15.5 %

Table 2. Relative abundance (NPUE – in number of ind/m^2) of registered species by localities, ∑ - total NPUE by locality, mean- mean value of relative abundance for part Upper Zeta – Nikšić Valley

NPUE	***S. labrax***	***P. phoxinus***	***S. cephalus***	∑
Brezovik	0.032	0.645	0.064	**0.741**
Duklov most	0.027	0.928	0.176	**1.131**
Mean	**0.029**	**0.787**	**0.120**	**0.936**

b)Biomass

During the survey period, the highest CPUE and absolute biomass were recorded for Squalius cephalus (Table 3 and 4).

Table 3. Relative biomass (CPUE – gr/m^2) of registered species by localities, ∑ - total CPUE by locality, mean-mean value of relative abundance for part Upper Zeta – Nikšić Valley

CPUE	***S. labrax***	***P. phoxinus***	***S. cephalus***	∑
Brezovik	2.539	1.523	5.557	**9.619**
Duklov most	0.597	2.363	16.629	**19.589**
Mean	**1.568**	**1.943**	**11.093**	**14.604**

Table 4. Absolute biomass (kg/ha) of registered species by localities, ∑ - total biomass by locality, mean-mean value of absolute biomass for part Upper Zeta – Nikšić Valley

Biomass (kg/ha)	***S. labrax***	***P. phoxinus***	***S. cephalus***	∑
Brezovik	20.731	9.517	54.492	**84.740**
Duklov most	4.661	14.613	166.309	**185.584**
Mean	**12.696**	**12.065**	**110.401**	**135.162**

2.Lower Zeta-Bjelopavlići – upper part of the watercourse

a)Fish assemblage

Four species have been registered in the area of lower Zeta – Bjelopavlići, upper part of watercourse (Table 5). During the survey period, the highest relative abundance was recorded for Barbus rebeli and Salmo farioides (Table 6).

Table 5. Registered species, No-number of individuals; %-percentage in the sample by locality

LOWER ZETA BJELOPAVLIĆI – UPPER PART OF WATERCOURSE			
Locality	**Species**	**No**	**%**
Glava Zete	*Salmo farioides*	19	*100 %*
Ostrvo	*Salmo farioides*	9	*45 %*
	Barbus rebeli	8	*40 %*
	Salmo marmoratus	3	*15 %*
Tunjevo	*Salmo farioides*	7	*9 %*
	Barbus rebeli	16	*20 %*
	Telestes montengrinus	57	*71 %*

Table 6. Relative abundance (NPUE – in number of ind/m^2) and of registered species by localities, ∑ - total NPUE by locality, mean-mean value of relative abundance for part Lower Zeta Bjelopavlići – upper part of the watercourse

NPUE	***S. farioides***	***B. rebeli***	***S. marmoratus***	***T. montenigrinus***	∑
Glava Zete	0.096				**0.096**
Ostrvo	0.048	0.043	0.016		**0.107**
Tunjevo	0.037	0.085		0.304	**0.427**
Mean	**0.060**	**0.064**	**0.016**	**0.304**	**0.210**

b)Biomass

During the survey period, the highest CPUE and absolute biomass was recorded for *S. farioides* (Table 7 and 8).

Table 7. Relative biomass (CPUE – gr/m^2) of registered species by localities, ∑ - total CPUE by locality, mean- mean value of relative abundance for part Lower Zeta – Bjelopavlići, the upper part of the watercourse

CPUE	***S. farioides***	***B. rebeli***	***S. marmoratus***	***T. montenigrinus***	∑
Glava Zete	14.355				**14.355**
Ostrvo	5.379	2.883	4.600		**12.862**
Tunjevo	2.847	4.557		7.488	**14.892**
Mean	**7.527**	**3.720**	**4.600**	**7.488**	**14.036**

Table 8. Absolute biomass (kg/ha) of registered species by localities, ∑ - total biomass by locality, mean- mean value of absolute biomass for part Lower Zeta – Bjelopavlići, the upper part of the watercourse.

Biomass (kg/ha)	*S. farioides*	*B. rebeli*	*S. marmoratus*	*T. montenigrinus*	∑
Glava Zete	191.400	0	0	0	**191.400**
Ostrvo	69.240	30.178	51.450	0	**150.868**
Tunjevo	37.540	50.289	0	98.867	**186.696**
Mean	**99.393**	**26.822**	**17.150**	**32.956**	**176.321**

3.Lower Zeta – Bjelopavlići – the lower part of the watercourse

a)Fish assemblage

13 species have been registered in the area of lower Zeta – Bjelopavlići, the lower part of the watercourse (Table 9).

Table 9. Registered species, No-number of individuals; %-percentage in the sample by locality

LOWER ZETA BJELOPAVLIĆI – LOWER PART OF THE WATERCOURSE			
Locality	**Species**	**No**	**%**
Danilovgrad	*Salmo farioides*	4	2 %
	Barbus rebeli	6	2.5 %
	Telestes montenegrinus	26	11 %
	Phoxinus sp.	115	51 %
	Anguilla anguilla	3	1.5 %
	Alburnus scoranza	42	18 %
	Salmo marmoratus	2	1 %
	Rutilus prespensis	26	11 %
	Squalius platyceps	6	2.5 %
Pričelje	*Barbus rebeli*	5	1 %
	Squalius platyceps	7	2 %
	Phoxinus sp.	187	48 %
	Anguilla anguilla	4	1 %
	Alburnus scoranza	61	16 %
	Chondrostoma ohridanum	4	1 %
	Cobitis ohridana	19	5 %
	Rutilus prespensis	50	13 %
	Telestes montengrinus	49	13 %
Vranjske njive	*Salmo farioides*	5	1.5 %
	Barbus rebeli	10	3 %
	Telestes montengrinus	22	6 %
	Phoxinus sp.	314	88 %
	Anguilla anguilla	1	0.5 %
	Squalius platyceps	2	0.5 %
	Salmo marmoratus	1	0.5 %

During the survey period, the highest relative abundance was recorded for *P. phoxinus* (Table 10).

Table 10. Relative abundance (NPUE – in number of ind/m^2) and of registered species by localities, ∑ - total NPUE by locality, mean-mean value of relative abundance for part Lower Zeta Bjelopavlići – the lower part of the watercourse.

NPUE	*S. farioides*	*B. rebeli*	*S. platyceps*	*P. phoxinus*	*A. anguilla*	*A. scoranza*	*C. ohridanum*	*S. marmoratus*	*C. ohridana*	*R. prespensis*	*T. montenigrinus*	∑
Danilovgrad	0.027	0.040	0.173	2.859	0.027	0.280		0.013		0.087	0.333	**3.839**
Pričelje		0.033	0.313	1.249	0.027	0.387	0.020		0.127	0.333	0.472	**2.961**
Vranjske njive	0.033	0.067	0.093	2.093	0.007			0.007			0.153	**2.453**
Mean	**0.030**	**0.047**	**0.193**	**2.067**	**0.020**	**0.333**	**0.020**	**0.010**	**0.127**	**0.210**	**0.853**	**3.91**

b)Biomass

During the survey period the highest CPUE was recorded for A. scoranza and *T .montenigrinus* (Table 11).

Table 11. Relative biomass (CPUE – gr/m^2) of registered species by localities, ∑- total CPUE by locality, mean-mean value of relative abundance for part Lower Zeta – Bjelopavlići, the lower part of the watercourse.

CPUE	*S. farioides*	*B. rebeli*	*S. platyceps*	*P. phoxinus*	*A. anguilla*	*A. scoranza*	*C. ohridanum*	*S. marmoratus*	*C. ohridana*	*R. prespensis*	*T. montenigrinus*	∑
Danilovgrad	3.853	1.895	7.620	1.362	6.420	7.107		5.214		2.867	7.343	**43.681**
Pričelje		1.437	8.687	4.030	6.420	9.407	3.543		0.796	9.533	10.550	**54.403**
Vranjske njive	4.510	3.417	1.787	4.093	1.677			2.513			3.093	**21.091**
Mean	**4.182**	**2.250**	**6.031**	**3.162**	**4.839**	**8.257**	**3.543**	**3.864**	**0.796**	**6.200**	**6.996**	**39.725**

The highest apsolute biomass was recorded for *T. montenegrinus*, *A. scoranza* and *S. platyceps*. (Table 12).

Table 12. Absolute biomass (kg/ha) of registered species by localities, ∑ - total biomass by locality, mean- mean value of absolute biomass for part Lower Zeta – Bjelopavlići, the lower part of the watercourse.

Biomass (kg/ha)	*S. farioides*	*B. rebeli*	*S. platyceps*	*P. phoxinus*	*A.anguilla*	*A. scoranza*	*C. ohridanum*	*S. marmoratus*	*C. ohridana*	*R. prespensis*	*T. montenigrinus*	∑
Danilovgrad	37.427	15.411	77.960	17.720	53.600	71.113	0	43.467	0	58.833	73.433	**448.964**
Pričelje	0	13.200	104.967	30.479	59.380	99.127	35.113	0	7.687	94.833	88.693	**533.479**
Vranjske njive	44.107	28.133	30.673	42.848	16.787	0	0	20.956	0	0	39.187	**222.690**
Mean	**27.178**	**18.915**	**71.200**	**30.349**	**43.256**	**56.747**	**11.704**	**21.474**	**2.562**	**51.222**	**67.104**	**401.711**

Data presented in this paper are the first one related to the entire Zeta River regarding the fishes resources in terms of absolute and relative biomass. Therefore, it is clear that there are no previous ones that could be used for comparison or determining any trend of the species abundance or biomass. During this investigation, 14 fish species were registered, out of a total of 20 that are reported in the literature (Marić, 2018). In terms of the composition of the fish fauna, the upper course of the Zeta River through the Nikšić Valley, as well as the upper course of the same river after the sinkhole and reappearance in the Bjelopavlići Valley, have a similar salmonid character (Table 1 and Table 5). In the upper part along the Bjelopavlići Valley, the fauna is somewhat richer and

consists of 4 species, two of which belong to trout species, *Salmo farioides*-brown trout and *Salmo marmoratus* – marble trout (Table 1 and Table 5). In both parts, in terms of abundance, cyprinid species are dominant (Table 2 and Table 6), while in terms of biomass the situation is different, in upper parts of Zeta river in the Bjelopavlić Valley brown trout is dominant (Table 4 and Table 8).

As for the lower course of the Zeta river through the Bjelopavlići Valley, this river sector showed the highest diversity of detected fish species, 13 of theme (Table 9). As it was expected, in this sector cyprinid species were dominant pointing to the cyprinid character (Table 9). Regarding their abundance and biomass, the same group of species showed their dominance where the most dominant were *S. platiceps, T. montenigrinus, R. prespensis* and *A. scoranza* in both abundance and biomass (Table 10 and Table 12).

From the data presented in this paper it is obvious that we are facing dramatically low abundance and biomass of salmonid species (personal experience). What is of highest concern is that we haven't detected soft muzzle trout (*Salmo obtusirostris*) in lower Zeta which was in 2012 lastly reported for Zeta River (Mrdak *et al*., 2012). The absence of this species in our samples implies the further decline of its population in Zeta River. This data imply that illegal fishing, using of forbidden tools as well as no adequate management and absence of the ranger service or fish guards motivate poachers to continue or even to become more frequent on this river. According to IUCN Red List, the soft muzzled trout *S. obtusirostris* is considered Endangered B2ab(v) throughout its dispersal area (IUCN 2021), while in the national legislation of Montenegro, this is the only protected fish species. During 1990's, their population sizes dramatically decreased in the whole dispersal area and in a few reports on monitoring of protected animal species in Montenegro it was reported that this species is the most probably extinct there (National Agency for Nature Protection of Montenegro, 2003, 2004 and 2005). However, one population at the locality of the village of Tunjevo with an ultimately small number of specimens was recorded (Sušnik *et al*. 2007; Mrdak *et al*. 2012).

Neither comprehensive monitoring of fish stocks nor the estimation of maximum sustainable yield on water bodies in Montenegro has ever been performed in the past. Only recently, through the CSBL projects (Conservation and Sustainable Use of Biodiversity at Lakes Prespa, Ohrid and Shkodra/Skadar) in period 2013 to 2018, has the monitoring of fish populations been established on Skadar Lake but, unfortunately, that process did not continue beyond the end of this project (Mrdak *et al*., 2017 b). For that reasons the presented result provide first and important data about diversity and biomass of fish fauna of the important Zeta River which is part of Lake Skadar water catchment area. The data confirms that Zeta River is an important fishery area in Montenegro. Taking in mind that Zeta watercourse in Bjelopavlići valley being proclaimed as Park of Nature the results of this paper should represent the basis for establishing long-term monitoring of fish fauna.

The management and sustainable use of the fish resources poses manifold challenges to competent authorities. First of all, vulnerable species such as trout species are protected under national and EU nature conservation legislation and require special conservation efforts. Second fishes are one of four indicators that determine the ecological status of water bodies according to the EU Water Framework Directive. That means that fish fauna (and other biota) under nearly undisturbed conditions, has to be maintained or restored, and that specific measures are to be taken to fulfill this requirement. The following are recommended for preparation management plan, implementation, and conservation of fish biodiversity in the Zeta River:

1. Banning or controlling destructive fishing gears
2. Identification of the fish breeding and nursery grounds and their protection as fishing no go zones
3. Overall public awareness should be expanded through training programs to restore the habitat of these valuable fish species from close extinction
4. Banning of fishing marble trout and soft-mouth trout, control of restaurants and banning of possession and sale of marble trout and soft-mouthed trout.
5. Establishment of breeding center for indigenous trout species.

CONCLUSION

Taking in mind the river Zeta watercourse in Bjelopavlići Valley was proclaimed as a Park of Nature, the present investigation generate first, very important and comprehensive data set. The presented results are the first one related to the entire Zeta River regarding the fish's resources in terms of absolute and relative biomass. Unfortunately data confirms that we are facing dramatically low abundance and biomass of salmonid species which indicates that repopulation measures should be implemented urgently.

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Short Communication

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A GRAFTING-INDUCED SORGHUM-MAIZE HYBRID

SUMMARY

Interspecific grafting is limited by graft-incompatibility but regeneration of hybrid plants from grafting of genetically distinct scions and rootstocks has been reported in tobaccos. Here we report the regeneration of a hybrid plant via grafting between cross-incompatible sorghum and maize. By grafting maize and sorghum plants and regenerating hybrid from the graft junction in selection media, we generated a plant that was phenotypically a hybrid between sorghum and maize, i.e., the hybrid produced a seed-bearing "ear" toward the top of the stalk. To our knowledge, this is the first report of successful hybrid from grafting between two monocot plants.

Keywords: sorghum, maize, grafting, regeneration, hybrid

INTRODUCTION

Grafting is a common horticulture technique that has been in practice for over 2000 years. Use of grafting has been referenced in Bible, ancient Greek and Chinese text, indicating its practice by at least 5th century BCE (Melnyk and Meyerowitz 2015). Grafting has been important in agriculture and horticulture for centuries and some of the major benefits from grafting include domestication of woody fruit plants such as apples, pears, and plums (Mudge *et al*. 2009), asexual propagation of desirable plants as well as introduction of resistance to various biotic and abiotic stresses (Lee *et al*. 2010) and changes in growth habits of scion by altering its characteristic such as size, growth vigor, and fruit yield (Lee *et al.* 2010, Mudge *et al*. 2009).

Similar to sexual hybridization, there is also graft incompatibility between species. In sexual hybridization, due to reproductive barriers interspecific hybrids between cross-incompatible parents are not viable because of gametic

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incompatibility (prezygotic) and hybrid breakdown (postzygotic incompatibility) (Bates and Deyoe 1973). Similarly, there is also grafting-incompatibility, i.e., majority of plants will graft to themselves, fewer will graft to very closely related species, and only rarely will plants successfully graft to more distant relatives (Melnyk and Meyerowitz 2015). In eudicot compatible grafting at graft junction, ruptured cells collapse and the intact cells adhere to the opposing tissue right after grafting. Subsequently, cell divides to give rise to phloem and xylem, followed by the formation of plasmodesmata and cytosolic channels between cells across the junction. In eudicot incompatible grafting, cell still divides at the junction but phloem and xylem differentiation may not occur. In both cases, aligning vascular cambia between scions and rootstocks improves grafting success rate (Melnyk and Meyerowitz 2015).

Although grafting in eudicots is common and has been in use for centuries, grafting between monocots is more difficult as monocots have scattered vascular bundles and do not have a vascular cambium, but can be grafted by aligning meristem tissues from scion and rootstock although success rate may still be low (Muzik and La Rue 1952, 1954). This explains little research reported in the literature dealing with monocot grafting, if any they focus on greenhouse setting grafting. Muzik and La Rue (1952, 1954) had a 3% graft success rate while grafting monocot plants. Successful grafts generated between para grass (*Panicum purpurascens* Raddi.) and merker grass (*Pennisetum purpureum Schum*. var. *merkeri*) grew and set seeds. However, grafting of monocots in tissue culture setting has not been reported. Monocots such as maize, rice, and sorghum are some of the economically most important crops. Grafting of such commercially important plants to improve their agronomic traits might in long term be economically more beneficial. Successful grafting although less frequent could provide monocot plants with genetic variation that will help improve traits such as growth rate, size, yield, environmental stress tolerances and more.

Grafting involves the physical connection between the shoot of one plant (scion) and the rooted part of another (rootstock) plant. After grafting, plants respond rapidly by activating the wound healing and begin the regeneration process (Melknyk, 2017). The wound healing process might be activated by the disconnection between leaves and the roots by changing the transport dynamics (Friml and Palme 2002) or by detection of damages to cell in the graft region and subsequent triggering of the plant defense and growth responses (Nushe, 2012). Graft healing leads to regeneration of tissues around the wound. Normally, grafting creates a compound genetic system by uniting two or more distinct genotypes, each of which maintains its own genetic identity throughout the life of the grafted plant (Mudge *et al*. 2009), but the closeness of cells from the two genotypes allows movement of nuclei through plasmadamata in a cytomixis-like process (Fuentes *et al.* 2014). This produces hybrid cells with chromosomes from both scion and rootstocks and hybrid plants after regeneration. This process can serve as a route to generate asexual allopolyploid hybrids (Fuentes *et al*. 2014, Stegemann and Brock 2009, Stegemann *et al*. 2012).

Grafting to produce new species of plant is relatively new. Although allopolyploidization in plants is common and it leads to success of crop domestication as well as speciation and environmental adaptation, cross species allopolyploidization through asexual mechanism is very rare. In tobacco, the grafting between herbaceous (*Nicotiana tabacum*) and woody (*Nicotiana glauca*) tobaccos produced hybrid plant from graft junction which had the genomes from both rootstock and scion and was named *Nicotiana tabauca*. The allopolypoid hybrid was a result of migration of nuclei from cell to cell at graft junction through plasmodesmata in a cytomixis-like process, not through cell fusion (Fuentes *et al.* 2014). The movement of the entire nuclear genome across the graft junction thus raises the possibility of generating new plant species which has the characters of both parents and might lead to the generation of economically important hybrid plants. In this study, we demonstrated that graft-induced hybrids can be produced between monocot scions and rootstocks.

MATERIAL AND METHODS

Plant materials: Surface sterilized transgenic maize (resistant to phosphinothricin) and sorghum (resistant to hygromycin) seeds were grown under aseptic conditions by germinating in Magenta® box with MS Medium supplemented with 3% sucrose. Plants were grown under diurnal cycle of 16 hours light and 8 hours of dark at 25°C.

Grafting: grafting experiments were performed using sterile transgenic plants under aseptic condition in the laminar air flow hood. Stems of similar sized transgenic maize and sorghum plants were cut at approximately 45-degree angle. The scion and rootstock were joined and held together using sterile silicon tubes. The reciprocal grafting was done with each plant serving as both rootstock and scion, giving rise to two grafts. These grafts were grown in MS media supplemented with 3% sucrose and 1 mg/L 2,4-D (2, 4- dichlorophenoxyacetic acid) for 2 weeks at 25°C with 16 hrs light and 8 hrs dark photo period.

Selection and regeneration of hybrid: After 2 weeks, graft site was excised and exposed to regeneration medium containing 50 mg/l hygromycin and 3 mg/l phosphinothricin. The regeneration medium was also supplemented with 3.5 mg/l BAP, 0.2 mg/l IBA and 0.2 mg/L kinetin. Successful selection was defined as the growth of callus followed by production of shoots from the graft region. Plants so produced were transferred to regeneration medium with double selection (phosphinothricin and hygromycin) to produce longer shoots and roots.

Putative hybrid plant was transferred to soil after regeneration of roots in regeneration media followed by 3 days of gentle acclimatization to open air. The plants in soil were transferred to greenhouse and grown at 30°C with natural daylight with periodic watering and fertilization.

RESULTS AND DISCUSSION

Over 850 grafts were made between transgenic sorghum and transgenic maize plants in aseptic conditions. Reciprocal cleft grafting was used in the

majority of grafts; however, in some cases where maize stem size was bigger than sorghum, maize was used as the rootstock and sorghum as scion (Figure 1).

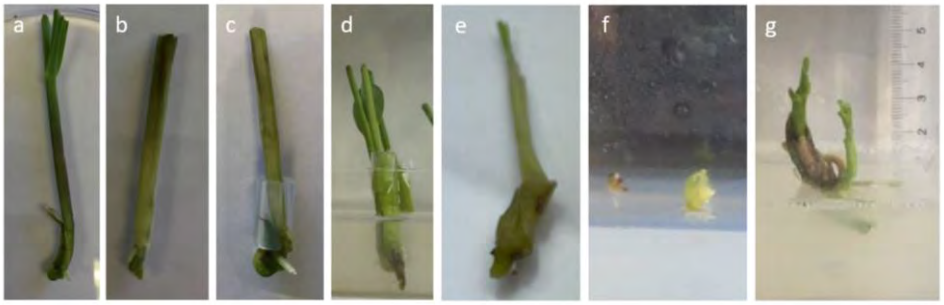

Figure 1. Different stages of monocot grafting in vitro: (a) Maize (b) Sorghum (c) Graft with tube (d) Graft in Ms media day 1 (e) Graft after 15 days tube removed (f) graft in regeneration for 3 weeks (g) Graft in regeneration for 5 weeks

Silicon tubes pre-sterilized in bleach were used to hold the grafted plants in place and they worked better than paper clips or copper wire and were easy to use. After 2 weeks of incubation in MS media supplemented with 2,4-D, graft junctions were cut in sterile environments and transferred to selection media. A total of 358 graft junctions (42% of 850) that were properly connected and still living were transferred to selection media. In the following two weeks the graft junctions were under double selection of hygromycin and phosphinothricin in which 205 of them died. The 153 surviving graft junctions were transferred to regeneration media with double selection. Regeneration media was changed every 3 weeks to supply fresh nutrients and selection. Following multiple subcultures on regeneration media the number of surviving graft junction with calli was reduced to 30. From these only 6 plantlets (0.59%) regenerated and grew---we also used germinating sorghum and maize seeds for grafting as in Reeves et al. (2022) but failed to generate any hybrids (data not shown). These six plantlets were transferred to rooting media with double selection, on which 2 more died. Out of remaining four, two were able to produce the roots and thus transferred to soil following acclimatization for 3 days and grown in greenhouse. One plant died after 2 weeks whereas other one started to grow. Other two plantlets in rooting media were subcultured multiple times but died eventually.

The putative hybrid plant looked like sorghum at first: smooth green leaves with a thin stem. But growth stalled after 3 weeks and after another week a new stem started to grow. This time the plant started to show leaf features of maize plant while the original stem died off. After a month the plant was around 10 cm tall. We noticed a seed-bearing ear growing in the stalk toward the top of the plant. This indicated the plant although very small was mature enough to produce a “corn ear” as part of the stalk, not as “ears” on the stalk (Figure 2).

Figure 2. Hybrid plant at different growth stages: (a) Hybrid in soil at week 1. (b) Hybrid in soil at week 4. (c) Hybrid at week 7.

Mobility of DNA between scions and rootstocks in graft junction has been demonstrated in recent years. For example, it has been demonstrated that plant grafting can result in the exchange of genetic information via either large DNA pieces or entire plastid genomes (Stegemann and Bock 2009), complete chloroplast genomes can travel across the graft junction from one species into another (Stegemann *et al*. 2012) and complete nuclear genomes can travel across the graft junction from one species into another (Fuentes *et al.* 2014). From these studies, the group were able to generate graft-induced hybrids (Fuentes *et al.* 2014). Fuentes *et al.* (2014) were the first to report hybrid plant (*Nicotiana tabauca*) that combined nuclear genomes of *N. glauca* and *N. tabacum*. These results indicate the transfer of nuclear genomes across the graft junction that produces hybrids.

In vitro grafting successes of under 1% in our study although lower than expected is significant in the sense that we managed to grow it from the graft junction between two monocot plants. This implies the transfer of genomic DNA between two plants that produced a new hybrid. This hybrid plant being resistant to both hygromycin and phosphinothricin demonstrates the presence of DNA from both maize and sorghum genomes, although the plant was much smaller than its scion/rootstock parents (Figure 2) due to hybrid weakness (Chen *et al*. 2014). The hybrid plant had a seed-bearing ear close to the position of maize tassel or sorghum panicle. This may be due to subgenome dominance of maize in the hybrid as demonstrated in allotetraploid *Senecio mohavensis* (Alexander-Webber *et al*. 2016) in which the allopolyploid hybrids preferentially express genes from one parent with corresponding phenotypic consequences. In our hybrid, the maize genome may have a gene expression bias in its favor although this is to be confirmed. By producing the hybrid from the graft junction in vitro we demonstrated in this study that different species of monocot plants not only can be grafted but also their nuclear genomes be transferred through graft

junction to produce a hybrid. It also showed that the sexually incompatible sorghum and maize (Bernard and Jewell 1985) are graft-compatible although with the characteristic low success rate of monocots.

CONCLUSIONS

In this study, we grafted phosphinothricin-resistant maize and hygromycin-resistant sorghum plants in vitro and regenerating hybrid from the graft junction in selection media. We generated a plant that was phenotypically a hybrid between sorghum and maize, i.e., the hybrid produced a seed-bearing "ear" toward the top of the stalk. To our knowledge, this is the first report of successful hybrid from grafting between two grass species.

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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.

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