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THE INFLUENCE OF FERTILISATION ON THE YIELD AND ANTIOXIDANT CAPACITY OF COMMON AND TARTARY BUCKWHEAT

SUMMARY

Buckwheat is an annual plant in the Polygonaceae family that is classified as a pseudocereal. It is mainly used as a source of antioxidants and gluten-free foods. Buckwheat yield and quality depend on genetic properties, environmental conditions, and cultural practices. Therefore, this study aimed to determine the effects of organomineral fertiliser on buckwheat yield and antioxidant activity in the environmental conditions of Bosnia and Herzegovina. The treatments used in this research consisted of a combination of fertilisers (fertilised and non-fertilised) and two buckwheat species (common and tartary buckwheat). During 2019, two field experiments were conducted in Butmir and Kakanj (B&H). The tested properties were plant height, a mass of 1000 kernels, yield, total phenolic, and antioxidant activity of leaves, flowers, and kernels. Yield depended on the treatment, ranging from 946.9 kg ha^{-1} to 1438.6 kg ha^{-1}. The total phenol contents were affected by fertiliser treatment, buckwheat species, and plant organs. The kernel had the lowest total phenol content (30.55 mg GAE g^{-1}), whereas the flowers had the highest content (158.03 mg GAE g^{-1}).

Keywords: tartary buckwheat, common buckwheat, organomineral, fertiliser, antioxidant capacity, yield

INTRODUCTION

Buckwheat is an annual plant in the Polygonaceae family that is classified as a pseudocereal. Although there are 23 different species of buckwheat in the world, only common (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn) are cultivate (Gavrić *et al.*, 2018). Buckwheat is mainly grown on all continents. On the other hand, tartary buckwheat is grown at higher altitudes, including in China, Bhutan, India, Nepal, Slovenia, and

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Luxembourg (Luthar *et al.*, 2020). Although both species of buckwheat have a similar morphology and growing practise, there are some differences. For example, tartary buckwheat, compared to common buckwheat has various advantageous traits, such as cold tolerance, self-pollination ability, and better kernel yield (Tsuji *et al.*, 2000). Buckwheat is commonly referred to as a pseudocereal. It is an excellent food source of nutrients for humans. The flour made from buckwheat is gluten free (Siqueira *et al.*, 2020) and contains a balanced amino acid composition, with arginine and lysine particularly abundant (Gavrić *et al.*, 2018; Nicolic *et al.*, 2019). Additionally, buckwheat flour has a higher fiber content than other cereals (Shruti *et al.*, 2015).

Because of buckwheat's positive impact on human health, buckwheat has recently grown in popularity in human nutrition. Several studies have demonstrated that buckwheat intake affects the treatment and prevention of numerous illnesses, including diabetes (Qiu *et al.*, 2016), cardiovascular disorders, arterial hypertension (Llanaj *et al.*, 2022), and cancer (Dziedzic *et al.*, 2018; Noreen *et al.*, 2021). The therapeutic properties of buckwheat are the result of its high antioxidant potential. Buckwheat's antioxidant potential is due to its high phenolic content (Guo *et al.*, 2011). Total phenols are found mainly in all plant organs (leaves, flowers, kernel, stem, root). Their content depends primarily on the species of buckwheat. Also, many environmental factors, including aridity, salinity, ultraviolet radiation, altitude, weather, etc., significantly affect the production of these components (Sharma *et al.*, 2019; Valle *et al.*, 2020). On the other hand, cultural practices directly alter the environment, affecting plant quality and productivity (Gadžo *et al.*, 2016). For example, one of the more frequent cultural practices is the application of fertilisers, which significantly affects the growth, development, yield, and quality of plants.

Buckwheat can be grown with small doses of organic and mineral fertilisers. Organic fertiliser positively impacts soil structure and contains all macro and micronutrients. However, the main disadvantages of organic fertilisers are their lack of nutrients and the slow decomposition of organic materials (Hirzel *et al.*, 2018; Yürürdurmaz, 2022). On the other hand, mineral fertilisers are available to plants and contain significant concentrations of macronutrients, allowing for excellent yields. However, applying these fertilisers, especially in high quantities, negatively affects the environment, including reduced soil microbial biodiversity and surface and subsurface water contamination (Timsina, 2018). Thus, both organic and mineral fertilisers have certain benefits and drawbacks. Recently, organomineral fertilisers are also used in plant cultivation. Organomineral fertilisers combine the advantages of organic and mineral fertilisers (Ojo, Olowoake and Obembe, 2014).

Additionally, a few authors' research (Corrêa *et al.*, 2016; Da Rosa *et al.*, 2022; Gavrić *et al.*, 2021) has shown that adding organomineral fertilisers to certain crops improves their growth. However, although the use has expanded considerably in some parts of the world, little has been studied in the environmental conditions of Bosnia and Herzegovina (B&H). In this context, hypothesis of this study is that the applied organomineral fertiliser will increase buckwheat yield and antioxidant activity. Therefore, this work aims to influence of organomineral fertilisers on buckwheat yield and antioxidant activity in the environmental conditions of B&H.

MATERIAL AND METHODS

Experiment location

Two field experiments (Kakanj and Butmir, B&H) (Figure 1) were conducted in 2019. The first field experiment was carried out in Kakanj, B&H (44°07'30.1 "N, 18°07'42.3"E; 400 m a.s.l.) at a private farm called Gavrić. The second field experiment was performed in Sarajevo, B&H, at the Faculty of Agriculture and Food Science (43°49'34.42" N, 18°19'18.48" E; 505 m a.s.l.).

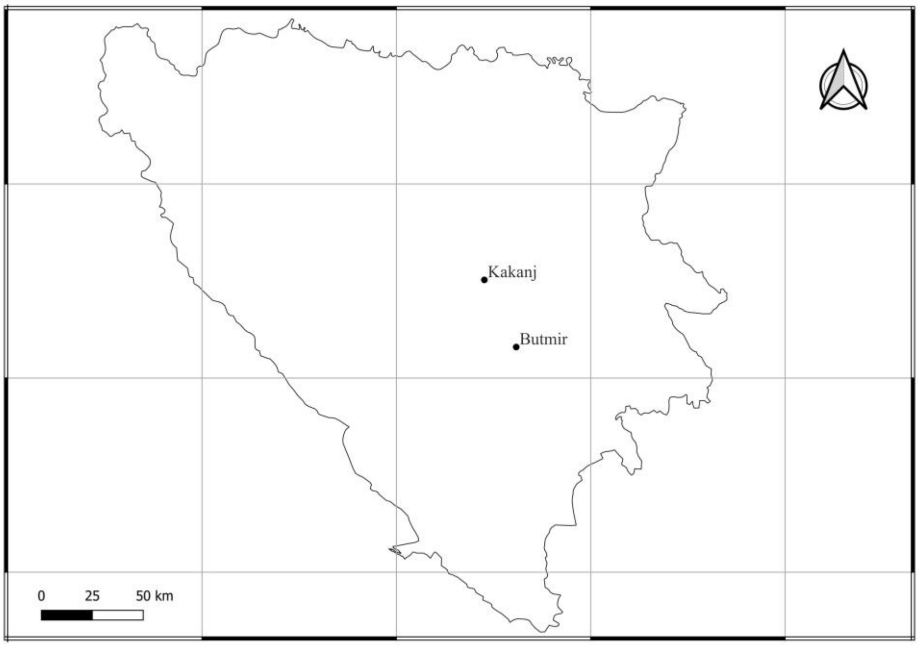

Figure 1. The locations of field experiments

Meteorological data sources

This research used data from two regional meteorological stations (Zenica and Butmir) of the Federal Hydrometeorological Institute, B&H (FHMZ, 2022). The study used data for the average monthly air temperature and precipitation amount. Figure 1 presents the climate data collected from regional meteorological stations. Mean monthly temperatures differed between the two study locations and the reference period (1961–1990). For example, the average temperature in Zenica (location Kakanj) during the growing season of 2019 (June–August) was 3.5°C warmer than it was reference period (1961–1990) (Figure 2). At the same study location, there was 58.7 mm more precipitation than in the reference period

(1961-1990). Similar weather were recorded at another location. The results demonstrate that Sarajevo (location Butmir) experienced above-average temperatures during the growing season. The average temperature ranged from 21.0 in July to 21.9 in August. During the same period (June to August), 234.6 mm of rain fell, about the same as the reference period.

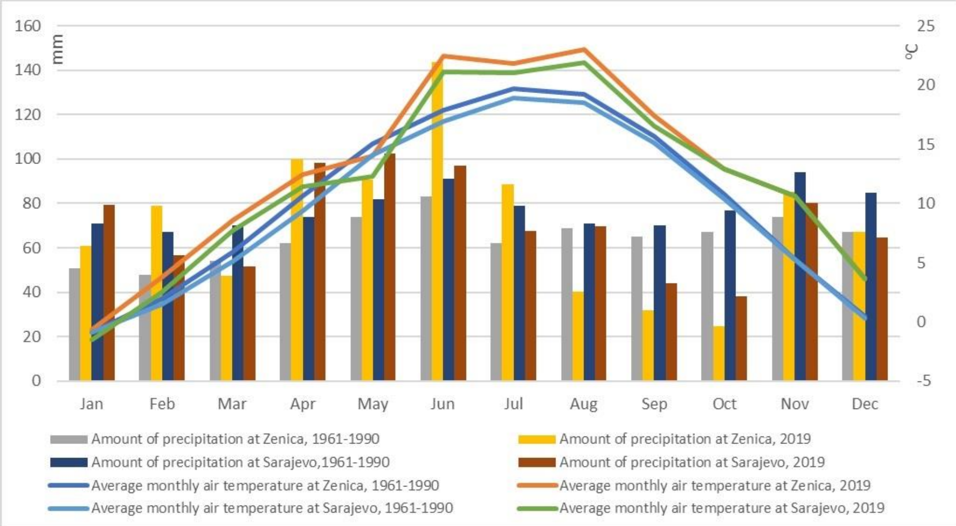

Figure 2. Average monthly temperatures and amount of rainfall

Treatments and experiment design

The treatments used in this research consisted of a combination of fertilisers (fertilised and non-fertilised) and two buckwheat species (common and tartary buckwheat). Common buckwheat (*Fagopyrum esculentum* Moench, cv. Darja) from Slovenia and tartary buckwheat (*Fagopyrum tataricum* Gaertn) from Luxembourg were used for sowing. Organomineral fertiliser (Humus 20 Goldfert) was applied at a rate of 250 kg ha^{-1}. The fertiliser contained organic matter (20%), N (10%), P_2O_5 (10%), K_2O (10%), and SO_3 (15%). The basic plot size was 4.8 m^2. The experimental plots were organised in a random block design with four replications. At both locations, the buckwheat was manually sown with 300 kernels per square metre on May 22, 2019. During the growing season, weeds were eliminated by hand. Buckwheat was harvested at the stage of technological maturity at both locations (September 10, 2019).

Soil analysis

Before the experiment set, soil samples were taken. The soil's pH was measured after the sample preparation using a digital pH metre (ISO 10390, 1994). It was used the dichromate method determines the humus content (ISO 14235, 1998). The Al method evaluated available P and K (Egnér, Riehm and Domingo, 1960). The experimental plots contained the following chemical properties, according to soil analysis: pH 7.5, 5.50% organic matter, P_2O_5=20.10 mg 100 g^{-1} and K_2O=31.40 mg 100 g^{-1} (Location Kakanj), and pH 6.40, 2.32%

organic matter, P_2O_5=6.04 mg 100 g^{-1} and K_2O=18.30 mg 100 g^{-1} (Location Butmir).

Plant height, yield and mass of 1000 kernels

All data were recorded during the harvesting period. The height (cm) of ten randomly chosen plants per plot was measured from the soil surface. The yield per hectare was calculated based on the yield per 1 m^2. The mass of 1000 kernels were determined by kernel weighing after harvesting.

Materials for analysis

The total phenol content and antioxidant activity of leaves, flowers, and kernels is determined. Leaves and flower samples were collected at the full flowering stage (July 19, 2019). Kernel samples are collected at the stage of ripeness. The samples were dried for 30 days at ambient temperature in a dim environment. After drying, the samples were ground to a size of 1 mm.

Preparation of extracts

The extract was made in the following way: In a 100 mL volumetric flask, 1 g of each dried and milled sample was added. After that, the volume of the flask was filled with 60% ethano and mixed. Following extraction, the entire volume was filtered through Blue Ribbon filter paper and analysed.

Determination of total phenol content

A modified Folin-Ciocalteu method was used to determine the total phenol concentration of the extracts (Gavrić *et al*., 2023). Briefly, 5 mL volumetric flask was filled with 0.5 mL of extract, blank, or standard. After that, 0.25 mL of Folin-Ciocalteu reagent was added to the volumetric flask, and the reaction proceeded. Than, 0.75 ml of 20% Na_2CO_3 was added, and the volume of the flask was filled to the mark with water. The absorbance at 765 nm was measured using a UV-VIS spectrophotometer (Ultrospec 2100 pro). Gallic acid equivalents were used to express the total phenol content (mg GAE 100 g^{-1}).

Determination of antioxidant activity

The total antioxidant capacity of buckwheat extract was determined using the FRAP (ferric reducing antioxidant power) method (Benzie *et al.*, 1996). In summary, 240 µL of H_2O, 80 µL of ethanol extract, blank or standard, and 2.080 L of FRAP reagent (0.3 M acetate buffer: 20 mM $FeCl_3.6H_2O$:10 mM TPTZ=10:1:1) were mixed in an Erlenmeyer flask. After 5 min of 37°C incubation, the absorbance at 595 nm was measured. The total antioxidant capacity was determined using a calibration curve that was created using an aqueous solution of $FeSO_4.7H_2O$ as the standard.

Statistical methods

The research findings were statistically analysed using the SPSS 22 software. The average values of the determined data were evaluated using the Test Support at a significance level of p=0.05.

RESULTS AND DISCUSSION

The influence of fertiliser on plant height, the mass of 1000 kernels, and yield is presented in tables 1 and 2 and explained in detail below. The findings of the study indicated that the plant height, a mass of 1000 kernels, and yield of buckwheat in the control treatment were significantly lower (102.8 cm, 18.8 g, and 1092.6 kg ha^{-1}, respectively) than in the fertiliser treatment (110.2 cm, 19.6 g, and 1192.8 kg ha^{-1}, respectively). The application of organomineral fertilisers had a positive effect on the growth and productivity of buckwheat. This result was expected because the plant received additional nutrients for growth from the used fertiliser. Many authors (Katar *et al*., 2022; Sazhina *et al.*, 2020) have found a similar influence of fertilisers on the development of buckwheat. However, some authors also found that fertilisation can reduce yield. For example, Fang et al. (2018) recorded that increasing nitrogen fertiliser application from 0 to 45 kg ha^{-1} increased grain yield, but a further increase in the fertiliser dose decreased the grain yield. Yield reduction is associated with crop lodging (Gavrić *et al.*, 2018). Namely, buckwheat is known for its relatively high biomass productivity in different environmental conditions (Leto *et al.*, 2022), contributing to crop lodging (Gavrić *et al.*, 2018). Gavrić and Gadžo (2011) believe that buckwheat is an unpredictable crop in terms of fertilisers, and that poor soils and slow-degrading fertilisers should be preferred in the cultivation of this plant.

Table 1. Effect of fertiliser and buckwheat species on plant height, the mass of 1000 kernels, and yield

Organo-mineral fertiliser	**Species of buckwheat**	**Plant height**	**Mass of 1000 kernels**	**Yield**
		-cm-	-g-	-kg ha^{-1}-
Applied	Common buckwheat	129.3	23.7	1438.6
	Tartary buckwheat	91.1	15.5	946.9
	Average	**110.2a**	**19.6a**	**1192.8a**
Non-applied	Common buckwheat	119.9	22.9	1193.3
	Tartary buckwheat	85.6	14.7	991.8
	Average	**102.8b**	**18.8b**	**1092.6b**
Average	Common buckwheat	124.6a	23.3a	1315.9a
	Tartary buckwheat	88.3b	15.1b	969.1b
	Average	**106.5**	**19.2**	**1142.6**

Significant differences at the 0.05 level are indicated by different letters; ns indicate nonsignificant differences.

In our research, buckwheat species significantly impacted plant height, the mass of 1000 kernels, and yield. It was recorded that common buckwheat has higher values of research traits (124.6 cm, 23.3 g, and 1315.9 kg ha^{-1}, respectively) than tartary buckwheat (88.3 cm, 15.1 g, and 969.1 kg ha^{-1}, respectively) (Table 1). Therefore, our research recorded that a smaller mass of

1000 kernels caused a lower yield. Many studies (Da Rosa *et al.* 2022) have also shown that the mass of 1000 kernels is one of the main yield components strongly influencing buckwheat yield.

Table 2. Effect of location of research on plant height, the mass of 1000 kernels, and yield

Location of research	Plant height	Mass of 1000 kernels	Yield
	-cm-	-g-	-kg ha^{-1}-
Kakanj	117.4a	18.6b	1148.6ns
Butmir	95.5b	19.8a	1136.5ns
Average	106.5	19.2	1142.6

Significant differences at the 0.05 level are indicated by different letters; ns indicate nonsignificant differences.

The research location also significantly influenced the plant height and the weight of 1000 kernels (Table 2). Buckwheat cultivated at the Kakanj location had an 18.6% taller plant and a 6% lower mass of 1000 kernels than the Butmir location. The resulting differences are most likely the result of different weather conditions, that is, the distribution of precipitation between research locations. Namely, as seen in Figure 2, Kakanj location had more rainfall at the beginning of the growing season than the Butmir location. This rainfall positively affected plant growth. In contrast, the amount of precipitation at the period of kernel filling stage positively influences the mass of 1000 kernels. In our research, in the second part of the vegetation, i.e., the period of kernel filling stage, less precipitation was recorded at the Kakanj than at the Butmir (Figure 1). Several authors have also reported similar observations of weather effects on buckwheat plant height (Gavrić *et al.,* 2018; Ikanović *et al*., 2013; Popović *et al*., 2013). For example, Gavrić *et al.* (2018) found that plants were 11.5% lower in somewhat unfavourable weather conditions (lack of rain) than in favourable weather conditions.

The content of total phenols depended on all studied treatments. Thus, this component recorded significant differences in the leaves depending on fertilisation. (Table 3). The treatment without fertilisation had significantly higher phenol content (80.63 mg GAE g^{-1}) than the treatment with fertilisation (72.02 mg GAE g^{-1}). The use of fertilisation did not significantly affect the content of phenol in the flowers and kernels and the antioxidant activity in the leaves and flowers. These results follow Christensen *et al.* (2010), which found that different fertiliser rates have no significant effect on the level of phenolic compounds in all plant parts. In our research, the buckwheat species also significantly influenced the content of total phenols and antioxidant value (Table 3). It was found that tartary buckwheat has higher total phenols content and antioxidant capacity values.

Table 3. Effect of fertiliser and buckwheat species on total phenolic, flavonoid, and antioxidant capacity in leaves, flowers, and kernels

Organo-mineral fertiliser	Species of buckwheat	Total phenolics			Antioxidant capacity		
		Leaves	**Flowers**	**Kernels**	**Leaves**	**Flowers**	**Kernels**
			mg GAE g^{-1}			μM Fe^{2+} g^{-1}	
Applied	Common buckwheat	64.81	154.50	25.24	39.73	123.63	2.18
	Tartary buckwheat	79.23	164.38	34.77	53.89	118.55	23.92
	Average	**72.02b**	**159.44ns**	**30.01ns**	**46.81ns**	**121.09ns**	**13.05a**
Non-applied	Common buckwheat	78.08	144.08	25.60	45.40	117.18	3.05
	Tartary buckwheat	83.17	169.18	36.57	55.30	130.18	18.06
	Average	**80.63a**	**156.63ns**	**31.08ns**	**50.35ns**	**123.68ns**	**10.55b**
Average	Common buckwheat	71.45	149.29b	25.42b	42.57b	120.40ns	2.62b
	Tartary buckwheat	81.20a	166.78a	35.67a	54.59a	124.37ns	20.99a
	Average	**76.32b**	**158.03**	**30.55**	**48.58**	**122.38ns**	**11.80**

Significant differences at the 0.05 level are indicated by different letters; ns indicate nonsignificant differences. GAE-gallic acid equivalents.

The growing location also influenced the total phenolic content and antioxidant capacity. For example, buckwheat at location Kakanj had higher phenols content and antioxidant capacity than those at the Butmir location. These findings agree with previous research, which found that the total phenols were determined by the interaction of genetic factors and environmental conditions (Huda *et al.,* 2021; Ncube *et al*., 2012). Al-Huqail *et al.* (2020) believe that exposure of plants to stressful environmental conditions causes the synthesis of phenolics. Phenolics generally protect plants from unfavorable environmental conditions like UV radiation, drought, and extreme temperatures (Kumar *et al.*, 2014; Martínez-Silvestre *et al*., 2022). Therefore, their increased concentration is an indicator of stressful conditions (Stagnari *et al*., 2017). Stressful conditions were recorded in Kakanj location. Namely, as seen in Figure 1, the second part of the vegetation, i.e., during the collection period of samples, less precipitation was recorded at the Kakanj location than at the Butmir location.

The content of total phenols and antioxidant capacity were different in plant organs (Table 3 and 4). For example, in flowers, total phenol concentrations (158.03 mg GAE g^{-1}) were average, 5.17 times greater than in kernels (30.55 mg GAE g^{-1}). These findings are consistent with other researchers (Bystrická *et al*., 2011), who also reported that phenols concentration is different between the anatomical parts of buckwheat. However, data on the precise amount of total phenols in various plant organs varied greatly. These differences were likely due to differences in buckwheat species, cultivation techniques, climatic conditions, and extraction and measurement methods for total phenols. Nevertheless, the research results show that both buckwheat species are good sources of phenols and antioxidants.

Table 4. Effect of location of research on total phenolic, flavonoid, and antioxidant capacity in leaves, flowers, and kernels

Location of research	**Total phenolics**			**Antioxidant capacity**		
	Leaves	**Flowers**	**Kernels**	**Leaves**	**Flowers**	**Kernels**
	mg GAE g^{-1}			μM Fe^{2+} g^{-1}		
Kakanj	81.86a	184.31a	19.35b	53.29a	124.48ns	11.62ns
Butmir	70.79b	131.75b	41.74a	43.88b	120.29ns	11.98ns
Average	76.32	158.03	30.55	48.58	122.38	11.80

Significant differences at the 0.05 level are indicated by different letters; ns indicate nonsignificant differences. GAE-gallic acid equivalents.

Although our research has offered a glimpse into the impact of organomineral fertilisers in different environmental conditions on buckwheat productivity and antioxidant activity, they have certain limitations. There is not enough information about how organomineral fertiliser doses affect buckwheat's productive properties, crop lodging, and antioxidant properties. As a result, future studies should examine the potential impact of this fertiliser on these properties.

CONCLUSIONS

The experiment results showed that using organomineral fertiliser has increased buckwheat yield and antioxidant capacity. Therefore, using these fertilisers could be agrotechnical measure for the improvement buckwheat's mentioned characteristics. In addition, our research has shown that the leaves, flowers, and kernels of booth buckwheat species have a high level of total phenols and antioxidant activity, which is especially important if the plant is used as a medicinal plant.

Our study showed certain benefits that could be applied in practice. Specifically, organomineral positively impacted the yield of buckwheat. Therefore, the more intense application of these and similar fertilisers in practice could indirectly affect the increase in the yield and quality of buckwheat.

REFERENCES

Al-Huqail, A., El-Dakak, R. M., Sanad, M. N., Badr, R. H., Ibrahim, M. M., Soliman, D., & Khan, F. (2020). Effects of Climate Temperature and Water Stress on Plant Growth and Accumulation of Antioxidant Compounds in Sweet Basil (*Ocimum basilicum* L.) Leafy Vegetable. Scientifica, 2020. doi: 10.1155/2020/3808909

Benzie, I., & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidan power": the FRAP assay analytical biochemistry. Analytical Biochemistry.

Bystrická, J., Vollmannová, A., Kupecsek, A., Musilová, J., Poláková, Z., Čičová, I., & Bojňanská, T. (2011). Bioactive Compounds in Different Plant Parts of Various Buckwheat (*Fagopyrum esculentum* Moench.) Cultivars. Cereal Research Communications, 39(3), 436–444. doi: 10.1556/CRC.39.2011.3.13

Christensen, K. B., Kaemper, M., Loges, R., Fretté, X., Christensen, L. P., & Grevsen, K. (2010). Effects of Nitrogen Fertilisation, Harvest Time, and Species on the Concentration of Polyphenols in Aerial Parts and Seeds of Normal and Tartary Buckwheat (Fagopyrum sp.). European Journal of Horticultural Science, 75(4), 1611–4426.

Corrêa, J. C., Grohskopf, M. A., Nicoloso, R. da S., Lourenço, K. S., & Martini, R. (2016). Organic, organomineral, and mineral fertilisers with urease and nitrification inhibitors for wheat and corn under no-tillage. Pesquisa Agropecuaria Brasileira, 51(8), 916–924. doi: 10.1590/S0100-204X2016000800003

Da Rosa, J. A., da Silva, J. A. G., Vicenzi, R., Cecatto, A. P., Costa, A. R., Peter, C. L., Basso, N. C. F., Carvalho, I. R., Lautenchleger, F., Sgarbossa, J., Fraga, D. da R., & Bandeira, W. J. A. (2022). The density and sowing time of buckwheat in organic and conventional growing system. Australian Journal of Crop Science, 16(7), 941–948. Retrieved from https://search.informit.org/doi/10.3316/informit.731282554941232

Del Valle, J. C., Buide, M. L., Whittall, J. B., Valladares, F., & Narbona, E. (2020). UV radiation increases phenolic compound protection but decreases reproduction in Silene littorea. PLOS ONE, 15(6), e0231611. Retrieved from https://doi.org/10.1371/journal.pone.0231611

Dziedzic, K., Górecka, D., Szwengiel, A., Olejnik, A., Rychlik, J., Kreft, I., Drożdżyńska, A., & Walkowiak, J. (2018). The cytotoxic effect of artificially digested buckwheat products on HT-29 colon cancer cells. Journal of Cereal Science, 83, 68–73. doi: https://doi.org/10.1016/j.jcs.2018.07.020

Egnér, H., Riehm, H., & Domingo, W. R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. Kungliga Lantbrukshögskolans Annaler.

Fang, X., Li, Y., Nie, J., Wang, C., Huang, K., Zhang, Y., Zhang, Y., She, H., Liu, X., Ruan, R., Yuan, X., & Yi, Z. (2018). Effects of nitrogen fertiliser and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (*Fagopyrum esculentum* M.). Field Crops Research, 219, 160–168. doi: https://doi.org/10.1016/j.fcr.2018.02.001

FHMZ. (2022). Federal Hydrometeorological Institute Sarajevo. Retrieved from http://www.fhmzbih.gov.ba/latinica/index.php

Gadžo, D., Đikić, M., Oručević-Žuljević, S., Gavrić, T., & Grahić, J. (2016). Proizvodnja heljde u brdsko planinskim područjima-dosadašnja iskustva i budući izazovi. Simpozij Unapređenje Poljoprivrede, Šumarstva i Vodoprivrede u Kraškim, Brdskim i Planinskim Područjima-Racionalno Korištenje i Zaštita, ANUBiH.

Gavrić, T., Čadro, S., Gadžo, D., Đikić, M., Bezdrob, M., Jovović, Z., Jurković, J., Hamidović, S. (2018). Influence of meteorological parameters on the yield and chemical composition of common buckwheat (Fagopyrum esculentum Moench). The Journal Agriculture and Forestry, 64(4). doi: 10.17707/agricultforest.64.4.13

Gavrić, T., Gadžo, D. (2011). Yield and chemical composition of seeds of common buckwheat and tartary buckwheat by various cultivation technologies. Radovi Poljoprivrednog Fakulteta Univerziteta u Sarajevu (Works of the Faculty of Agriculture University of Sarajevo), 61(1), 7–17.

Gavrić, T., Jurković, J., Gadžo, D., Čengić, L., Sijahović, E., & Bašić, F. (2021). Fertiliser effect on some basil bioactive compounds and yield. Ciência e Agrotecnologia, 45. doi: https://doi.org/10.1590/1413-7054202145003121

Gavrić, T., Marković Stefan, & Čengić, L. (2023). Factibility of growing basil as an alternative crop for adaptation to climate change in Bosnia and Herzegovina. Chilean Journal of Agricultural Research, 83(1), 43–51. doi: 10.4067/S0718-58392023000100043

Guo, X.-D., Ma, Y.-J., Parry, J., Gao, J.-M., Yu, L.-L., & Wang, M. (2011). Phenolics content and antioxidant activity of tartary buckwheat from different locations. Molecules (Basel, Switzerland), 16(12), 9850–9867. doi: 10.3390/molecules16129850

Hirzel, J., Donnay, D., Fernández, C., Meier, S., Lagos, O., Mejias-Barrera, P., & Rodríguez, F. (2018). Evolution of nutrients and soil chemical properties of seven organic fertilisers in two contrasting soils under controlled conditions. Chilean Journal of Agricultural and Animal Sciences, 34(2), 77–88. doi: 10.4067/S0719-38902018005000301

Huda, M. N., Lu, S., Jahan, T., Ding, M., Jha, R., Zhang, K., Zhang, W., Georgiev, M. I., Park, S. U., & Zhou, M. (2021). Treasure from garden: Bioactive compounds of buckwheat. Food Chemistry, 335, 127653. doi: 10.1016/J.FOODCHEM.2020.127653

Ikanović, J., Rakić, S., Popović, V., Janković, S., Glamočlija, Đ., & Kuzevski, J. (2013). Agro-ecological conditions and morphoproductive properties of buckwheat. Biotechnology in Animal Husbandry, 29(3), 555–562.

ISO 10390. (1994). Soil Quality - Determination of pH. In International standard.

ISO 14235. (1998). Soil quality - Determination of organic carbon by sulfochromic oxidation. International Standard.

Katar, N., Katar, D., & Can, M. (2022). The Influence of Different Nitrogen Doses on Yield and Yield Components of Buckwheat (*Fagopyrum esculentum* Moench). Anadolu Tarım Bilimleri Dergisi, 37(3), 593–602.

Kumar, S., Sandhir, R., & Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Research Notes, 7(1), 1–9. doi: 10.1186/1756-0500-7-560/FIGURES/5

Leto, A., Hadžic, S., Vukotic, Dž., Salcinovic, A. (2022): Effects of different Pb and Cd concentrations on bioavailability in some plant species. Agriculture and Forestry, 68 (1): 285-296. doi:10.17707/AgricultForest.68.1.18

Llanaj, E., Ahanchi, N. S., Dizdari, H., Taneri, P. E., Niehot, C. D., Wehrli, F., Khatami, F., Raeisi-Dehkordi, H., Kastrati, L., Bano, A., Glisic, M., & Muka, T. (2022). Buckwheat and Cardiometabolic Health: A Systematic Review and Meta-Analysis. In Journal of Personalized Medicine 12 (12). doi: 10.3390/jpm12121940

Luthar, Z., Germ, M., Likar, M., Golob, A., Vogel-Mikuš, K., Pongrac, P., Kušar, A., Pravst, I., & Kreft, I. (2020). Breeding Buckwheat for Increased Levels of Rutin, Quercetin and Other Bioactive Compounds with Potential Antiviral Effects. Plants (Basel, Switzerland), 9(12). doi: 10.3390/plants9121638

Martínez-Silvestre, K. E., Santiz-Gómez, J. A., Luján-Hidalgo, M. C., Ruiz-Lau, N., Sánchez-Roque, Y., & Gutiérrez-Miceli, F. A. (2022). Effect of UV-B Radiation on Flavonoids and Phenols Accumulation in Tempisque (*Sideroxylon capiri* Pittier) Callus. Plants (Basel, Switzerland), 11(4). doi: 10.3390/PLANTS11040473

Ncube, B., Finnie, J. F., & Van Staden, J. (2012). Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. South African Journal of Botany, 82, 11–20. doi: 10.1016/J.SAJB.2012.05.009

Nikolic, O., Pavlovic, M., Dedic, D., Sabados, V. (2019): The influence of sow density on productivity and moisture in buckwheat grain (Fagopyrum esculentum Moench.) in condition of stubble sowing and irrigation. Agriculture and Forestry, 65 (4): 193-202. DOI:10.17707/AgricultForest.65.4.17

Noreen, S., Rizwan, B., Khan, M., & Farooq, S. (2021). Health Benefits of Buckwheat (Fagopyrum esculentum), Potential Remedy for Diseases, Rare to Cancer: A Mini Review. Infectious Disorders Drug Targets, 21(6), e170721189478. doi: 10.2174/1871526520999201224122605

Ojo, J. A., Olowoake, A. A., & Obembe, A. (2014). Efficacy of organomineral fertiliser and un-amended compost on the growth and yield of watermelon (*Citrullus lanatus* Thumb) in Ilorin Southern Guinea Savanna zone of Nigeria. International Journal of Recycling of Organic Waste in Agriculture, 3(4), 121–125. doi: 10.1007/s40093-014-0073-z

Popović, V., Sikora, V., Glamočlija, Đ., Ikanović, J., Filipović, V., Tabaković, M., & Simić, D. (2013). Influence of agro-ecological conditions and foliar fertilisation on yield and yield components of buckwheat in conventional and organic cropping system. Biotechnology in Animal Husbandry, 29(3), 537–546.

Qiu, J., Li, Z., Qin, Y., Yue, Y., & Liu, Y. (2016). Protective effect of tartary buckwheat on renal function in type 2 diabetics: a randomised controlled trial. Therapeutics and Clinical Risk Management, 12, 1721–1727. doi: 10.2147/TCRM.S123008

Sazhina, S., Plotnikov, A., Sozinov, A., & Porsev, I. (2020). Effect of organic fertilisers on buckwheat yield. BIO Web Conf., 27. Retrieved from https://doi.org/10.1051/bioconf/20202700093

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. In Molecules 24(13). MDPI AG. doi: 10.3390/molecules24132452

Shruti, P., Ey, Senthil, A., & Fatema, K. (2015). Effect of Hydrothermal Treatment on the Nutritional and Functional Properties of Husked and Dehusked Buckwheat. Journal of Food Processing and Technology, 6

Siqueira, V. C., Leite, R. A., Mabasso, G. A., Martins, E. A. S., Quequeto, W. D., & Isquierdo, E. P. (2020). Drying kinetics and effective diffusion of buckwheat grains. Ciência e Agrotecnologia, 44, 1–10.doi: 10.1590/1413-7054202044011320

Stagnari, F., Galieni, A., D'Egidio, S., Falcinelli, B., Pagnani, G., Pace, R., Pisante, M., & Benincasa, P. (2017). Effects of sprouting and salt stress on polyphenol composition and antiradical activity of einkorn, emmer and durum wheat. Italian Journal of Agronomy, 12(4), 293–301. doi: 10.4081/IJA.2017.848

Timsina, J. (2018). Can Organic Sources of Nutrients Increase Crop Yields to Meet Global Food Demand? Agronomy, 8(10), doi: 10.3390/agronomy8100214

Tsuji, K., & Ohnishi, O. (2000). Origin of cultivated Tatary buckwheat (*Fagopyrum tataricum* Gaertn.) revealed by RAPD analyses. Genetic Resources and Crop Evolution, 47(4), 431–438. doi: 10.1023/A:1008741513277

Yürürdurmaz, C. (2022). Impact of Different Fertilizer Forms on Yield Components and Macro–Micronutrient Contents of Cowpea (*Vigna unguiculata* L.). In Sustainability, 14(19). doi: 10.3390/su141912753

Radchenko, M., Trotsenko, V., Butenko, A., Masyk, I., Bakumenko, O., Butenko, S., Dubovyk, O., Mikulina, M. (2023): Peculiarities of forming productivity and quality of soft spring wheat varieties. Agriculture and Forestry, 69 (4): 19-30. doi:10.17707/AgricultForest.69.4.02

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PECULIARITIES OF FORMING PRODUCTIVITY AND QUALITY OF SOFT SPRING WHEAT VARIETIES

SUMMARY

Spring wheat becomes an important strategic grain crop in solving the problem of high–quality grain production. Therefore, main attention should be paid to selection of the most productive spring wheat varieties under certain conditions, as a variety is one of main means of increasing productivity of agricultural crops. The object of our research was varieties of soft spring wheat: Shirocco, Uliublena, Barvysta, Quintus. The best productivity indices of soft spring wheat were obtained when sowing variety Shirocco. This variety provided the largest plant weight –2.69 g, ear weight –1.51 g, and ear length –9.5 cm. The number of grains per ear was at the level of 25.0 pcs. with the mass of 1000 seeds –38.2 g. As a result of conducted research, it was found that yielding capacity of soft spring wheat averaged from 4.13 to 5.54 $t \cdot ha^{-1}$. The maximum grain yield on average during the research period was produced by variety Shirocco –5.54 $t \cdot ha^{-1}$. Varieties Uliublena, Barvysta, and Quintus provided grain yield at the level of 4.67, 4.13, and 4.81 $t \cdot ha^{-1}$, respectively. The highest amount of gluten, at the level of 32.5% with a protein content of 16.3%, was noted in the grain of soft spring wheat of variety Shirocco.

Keywords: variety, field germination, standing density, productivity, protein, gluten

INTRODUCTION

Grain industry is a source of stable development of agro-industrial complex. Despite the fact that soil and climatic conditions contribute to obtaining high yields, this industry does not meet export needs for high-quality grain. Spring wheat is becoming an important strategic grain crop in solving the problem of high-quality grain production (Yula, 2016).

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However, the area sown under spring wheat has decreased to 80–170 thousand hectares in recent years. Considering great importance of this crop, scientists recommend expanding sowing area for spring wheat to 1 million ha. The main reason of spring wheat acreage decrease is its low yielding capacity (3–3.5 $t.ha^{-1}$), while foreign and domestic varieties included in the state register have a potential yielding capacity of 5–8 $t.ha^{-1}$ (Melnyk *et al.*, 2006; Usov and Manko, 2015).

Spring wheat is characterized by increased demands for growing conditions, and this requires development of highly adapted varietal technologies for its growing. According to the results of Vlasenko (2006), it was found that an important condition for this is further improvement of technologies for growing grain crops, in particular, spring soft wheat. However, only modern high-yielding varieties can cover cultivation costs. Therefore, main attention should be paid to selection of the most productive varieties of spring wheat under certain conditions, as a variety is one of main means for increasing productivity of agricultural crops.

Analysis of crop yielding capacities over the past 20 years shows that biological potential of varieties is realized at the level of 40–75% (Sokolov, 2011). The decisive importance in increasing the yield of cereal grain crops belongs to modern varieties that have a high level of productivity, are adapted to certain growing conditions, are characterized by resistance to adverse abiotic factors of environment and have high grain quality (Yula, 2010). The maximum realization of productivity potential of domestic spring wheat varieties should be based on morphophysiological analysis and the creation of highly productive agrophytocenoses taking into account soil and climatic conditions (Yula and Drozd, 2020).

Realization of genetic potential of grain productivity and quality of modern spring wheat varieties is an important reserve for the growth of grain production (Holik and Kabatsiura, 2012; Xhulaj *et al*., 2022). Among the varieties of different ecological and geographical origin, the maximum yield will be provided only by those genotypes which are well adapted to the conditions of one or another soil-climatic growing zone in terms of their ability to form productivity and adaptability and fully correspond to it by their parameters. Revealing genetic potential of modern soft spring wheat varieties, which are adapted to specific soil and climatic zones, gives prospects for grain production of this crop (Ishchenko, 2021).

The issues of technology formation for spring wheat cultivation were studied in research of many authors. The most important are presented in the studies of Borysonik (2001), Kumakova (1988), Pidruchna (2000), Hrynyk (2000), Konovalov (1999), Radchenko *et al.* (2021). Despite the fact that this issue has been studied to a certain extent, the components of improving technology for growing spring wheat aimed at the maximum realization of the varieties' potential under conditions of northeastern forest-steppe of Ukraine are not fully disclosed.

MATERIAL AND METHODS

Research on the influence of varietal characteristics on productivity of spring soft wheat varieties was carried out during 2020–2022 at educational and scientific productive complex of Sumy National Agrarian University. The research field is located in the Sumy district of the Sumy region, Ukraine, geolocation data 50°52.742 N latitude, 34°46.159E longitude, 137.7 m above sea level (50°52'46.6"N34°46'07.8"E Map date ©2023 Google). The experiments were conducted according to the methods described by Dospekhov (1985), Pidoprygora and Pisarenko (2003).

The following varieties of spring wheat were the object of research: Shirocco, Uliublena, Barvysta, Quintus. In the experiment, the predecessor was soybean. Sowing was carried out at the onset of soil physical maturity at a temperature of 6–8°C to a depth of 3-4 cm by the usual way with a width between rows of 15 cm and a sowing rate of 5.0 million similar seeds per 1 ha, with the help of planter Klen –1.5. For pre-sowing cultivation, mineral fertilizers were applied in the form of nitroamophoska 200 kg ha^{-1} of physical weight. Nitroamofoska is a complex nitrogen-phosphorus-potassium fertilizer. Mass fraction of nitrogen (N) 16%, phosphorus (P) 16%, potassium (K) 16%. The form of fertilizer is granular. Total area of the plot is 50 m^2, accounting area –30 m^2, repetition of the experiment -three times. Placement of plots is systematic.

During phenological observations, the growth and development phase of spring wheat was considered to begin with appearance of at least 10% of plants, and to be completed with appearance of 75% of plants.

Dynamics of above-ground mass growth was determined in the main phases of growth and development by selecting 25 plants in typical locations of the plots in two incompatible repetitions. The structure of the harvest was determined by the method of sampling sheaves from each accounting area. The area of leaf surface was determined by calculation method. Amount of gluten was determined according to STST 13586.1–68 Grain. Methods of determining the quantity and quality of wheat gluten. Amount of protein was determined according to STST 10846–91 "Grain and the products of its processing". Protein determination method. Statistical processing of experimental data was carried out according to Dospekhov (1985) using Microsoft Excel.

The soil of experimental field is a typical heavy loamy and medium-humus black soil, which is characterized by the following parameters: humus content in arable layer (according to I.V. Tiuryn) is 4.0%, reaction of the soil solution is close to neutral (pH 6.5), the content of easily hydrolyzed nitrogen (according to I.V. Tiuryn) 9.0 mg, movable phosphorus and exchangeable potassium (according to F. Chyrikov) 14 mg and 6.7 mg per 100 g of soil, respectively.

The average daily annual air temperature in 2020 was 10.2 °C, which is 2.8 °C higher than the long-term index of 7.4 °C. The maximum temperature – 35.0 °C was recorded in July in the third decade, and the minimum temperature - minus 14.0 °C in the first decade of February. Precipitation amount for the reporting year was 466 mm, which is 127 mm less than the long-term norm (593 mm).The average daily annual air temperature in 2021 was 9.4 °C, which is 2.0 °C higher than the long-term index of 7.4 °C. The maximum temperature – 35.0 °C was recorded in June in the third decade, and the minimum temperature - minus 24.0 °C in the second decade of January. Precipitation amount for the reporting year was 453 mm, which is 140 mm less than the long–term norm (593 mm).

The average daily annual air temperature in 2022 was 8.7 °C, which is 1.3 °C higher than the long-term index of 7.4 °C. The maximum temperature – 36.0 °C was recorded in June in the third decade, and the minimum temperature - minus 18.0 °C in the second decade of January. Precipitation amount for the reporting year was 604 mm, which is 11 mm more than the long-term norm (593 mm).

The most favorable year for yield formation was 2022. In 2019 and 2020 were observed dry conditions, which were characterized by low precipitations and extreme fluctuations in air temperature during vegetation period.

RESULTS AND DISCUSSION

Seed germination is primarily influenced by soil moisture and temperature, as well as agrotechnical measures (Shevnikov, 2012). After sowing qualities of seeds, in particular such index as laboratory germination, field germination of seeds is practically the first real factor in the formation of crop productivity (Nazarenko *et al.,* 2021). In field conditions, a complex of factors simultaneously contribute to its increase or decrease, but the main ones are temperature and soil moisture (Sviderko *et al*., 2004). In field conditions, many factors affect seed germination at once, the influence of most of which cannot be calculated in isolated laboratory conditions. And isolation of each of the factors does not allow to learn real picture of factors interaction influence on the formation of shootings (Verhunov, 2000; Guedioura *et al*., 2023). In the conditions of the educational and scientific production complex of Sumy National Agrarian University, it was found that spring wheat varieties had different field germination. Thus, the highest field germination was obtained for variety Shirocco –94.0%, somewhat lower indices were obtained for varieties Quintus –90.6%, Barvysta –90.0% and Uliublena –88.6%. The density of standing ranged from 443 to 470 units/m^2. The highest density was recorded for variety Shirocco –470 pcs/m^2, and the lowest – for variety Ulyubena –443.0 pcs/m^2 (Table 1).

Preservation of plants during vegetation period was in the range of 88.9–94.5%. The highest preservation of plants was obtained by variety Shirocco – 94.5% (444.0 pcs m^2), and the lowest - by variety Uliublena –88.9% (394 pcs m^2). For variety Quintus, this index was recorded at the level of 92.1% (417.0 pcs m^2), for variety Barvysta –91.1% (410 pcs m^2) (Table.1). The highest yield of spring wheat is formed at a density of 400–500 pics m^2, this density is provided at the sowing rate 5.0–5.5 million of similar seeds per hectare after the best predecessors, and after the worst ones -5.5–6.0 million of similar seeds per hectare (Demydov *et al*., 2017).

Table 1. Standing density of soft spring wheat depending on varietal characteristics (average for 2020–2022)

Variety	Field germination, %	Standing density, pcs m^2	Preservation of plants during vegetation period	
			pcs m^2	%
Shirocco	94.0	470.0	444.0	94.5
Uliublena	88.6	443.0	394.0	88.9
Barvysta	90.0	450.0	410.0	91.1
Quintus	90.6	453.0	417.0	92.1
LSD_{05}	1.5	5.16	5.33	1.61

According to the results of the research, the highest coefficient of productive tillering of spring wheat plants was obtained in the Shirocco variety – 1.30, and the number of productive shoots was 577 pcs m^2. Somewhat lower indices were obtained for varieties Uliublena –1.28 (508 pcs m^2), Quintus –1.18 (490.7 pcs m^2), Barvysta –1.11 (453.3 pcs m^2). (Table. 2). The yield of cereal breads is determined by the number of ear-bearing stalks per area unit and productivity of their ears. Special attention is required to the question of influence of controlled factors on the development of stem systems, since they play a significant role in the formation of plant productivity. Spring wheat has low productive bushiness (Demydov *et al*., 2017).

Table 2. Coefficient of productive bushing and number of productive stems depending on varietal characteristics (average for 2020–2022)

Variety	Coefficient of productive bushing	Number of productive stems, pcs m^2
Shirocco	1.30	577.0
Uliublena	1.28	508.0
Barvysta	1.11	453.3
Quintus	1.18	490.7
LSD_{05}	0.03	9.15

For optimal photosynthesis, the crop must have a certain leaf surface area (Radchenko *et al.*, 2022). However, it is necessary to distinguish leaf surface as a means of accumulating plastic substances for yield formation of grain, roots, and various fruits. During formation of grain yielding capacity, the excess leaf surface will not contribute to high yield of the crop, as part of leaves will be shaded by its upper circles (Zhemela and Shevnikov, 2013). According to the results of Lozinska and Fedoruk (2017), the index of leaf surface area of spring wheat changed significantly depending on variety assortment, and the largest leaf surface area was formed in the earing phase in the range of 33.4–34.1 thousand m^2ha^{-1}. In the studies of Sumy National Agrarian University, it was found that in the tillering phase of spring wheat, the leaf surface area was 4.0-6.0 thousand m^2ha^{-1}. The largest area was obtained by variety Shirocco –6.0 thousand m^2ha^{-1}, leaf surface area of the rest of varieties decreased to 5.7 thousand m^2ha^{-1} – variety Quintus, to 4.5 thousand m^2ha^{-1} – variety Uliublena and up to 4.0 thousand m^2ha^{-1} – variety Barvysta (LSD_{05}=0.38). Corresponding regularity was also observed in the phase of tube forming and earing. Thus, the leaf surface area of variety Shirocco was 38.4 thousand m^2ha^{-1}, variety Quintus –35.6 thousand m^2ha^{-1}, variety Uliublena –29.4 thousand m^2ha^{-1}, variety Barvysta –25.7 thousand. m^2ha^{-1} (LSD_{05}=2.71).

In the experiment, the maximum area of leaf surface was noted in the earing phase. When sowing variety Shirocco, leaf surface area was the largest and amounted to 43.2 thousand m^2ha^{-1}, which is 7.2% more than leaf surface area of variety Quintus (40.1 thousand m^2ha^{-1}), 19.4% more than variety Uliublena (34.8 thousand m^2ha^{-1}) and 27.1% more than variety Barvysta (31.5 thousand m^2ha^{-1}) (LSD_{05}=2.54) (Fig. 1).

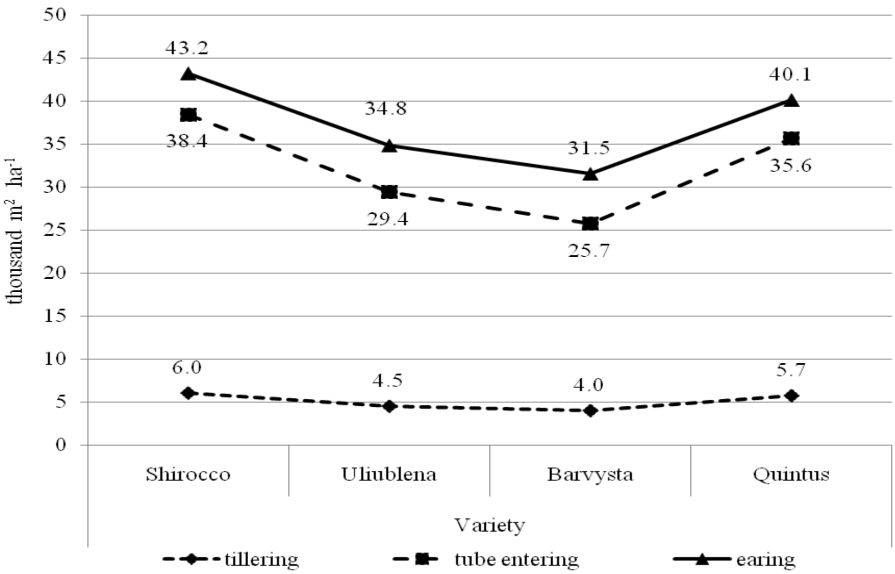

Figure 1. Leaf surface area of soft spring wheat by development phases depending on varietal characteristics (average for 2020–2022)

According to the results of research, the weight of a spring wheat plant varied between 2.05–2.69 g. The maximum weight of a plant was obtained for variety Shirocco –2.69 g. On average over the years of research, the plants of variety Shirocco had the largest ear weight –1.51 g. The lowest ear weight was obtained for variety Barvysta –1.25 g. For varieties Uliublena and Quintus, this index was 1.30 and 1.38 g., respectively (Table 3). The length of ear also varied depending on the variety. Thus, the length of ear for variety Shirocco is marked at the level of 9.5 cm, for variety Uliublena –8.0 cm, for variety Barvysta –7.8 cm, and for variety Quintus –8.8 cm (Table 3.). One of main components of plant productivity determining yielding capacity of grain crops is plant weight, ear weight, ear length. The formation takes place during the period when plants are optimally provided with light, moisture, heat and other vital factors (Kalenska and Shutyy, 2015).

Table 3. Weight of plant and ear, length of the ear of soft spring wheat depending on varietal characteristics (average for 2020–2022)

Variety	Weight of plant, g	Weight of ear, g	Length of ear, cm
Shirocco	2.69	1.51	9.5
Uliublena	2.25	1.30	8.0
Barvysta	2.05	1.25	7.8
Quintus	2.36	1.38	8.8
LSD_{05}	0.11	0.07	0.46

The maximum index of grain weight from an ear of spring wheat was obtained from variety Quintus -0.98 g, which is 2.0% more than from variety Shirocco (0.96 g), 6.1% more than from variety Uliublena (0.92 g) and 7.1% more than from variety Barvysta (0.91 g) (Table 4). The most important feature in increasing productivity according to the results of Lukianenko (1990) is the mass of grain from an ear, which is an important element of crop structure and must be taken into account when developing a variety model.

As a result of conducted research, it was found that the biggest number of grains in the ear of a spring wheat plant had variety Quintus –31.2 pcs., and the smallest - variety Barvysta –24.0 pcs. Varieties Shirocco and Uliublena provided the number of grains at the level of 25.0 and 24.7 pcs., respectively (Table 4). Graininess of the ear is an index of productivity, which in its turn depends on the length of the ear, the number of ears in the ear. That is why, studying of this trait is an important component in spring wheat cultivation (Shelepov *et al.,* 2004; Lukhovyd, 2023).

Yielding capacity level is determined both by number of seeds per plant and by weight of 1000 seeds (Domaratskiy *et al.,* 2022). The weight of 1000 seeds is a varietal characteristic, but it depends on the influence of various factors (Hryhoriv *et al.,* 2022).

The research varieties formed different weight of 1000 seeds. The difference in this index between the varieties was in the range of 6.8 g. The maximum weight values of 1000 seeds were obtained in the variant with variety Shirocco –38.2 g. Somewhat smaller values of this index were obtained in the variants with variety Barvysta –37.9 g and Uliublena –37.2 g, and the lowest weight of 1000 seeds was recorded for variety Quintus –31.4 g (LSD_{05}= 1.58) (Table 4).

Table 4. Structural indices of a soft spring wheat plant depending on varietal characteristics (average for 2020–2022)

Variety	Weight of grain from an ear, g	Number of grains in an ear, pcs.	Weight of 1000 seeds, g
Shirocco	0.96	25.0	38.2
Uliublena	0.92	24.7	37.2
Barvysta	0.91	24.0	37.9
Quintus	0.98	31.2	31.4
LSD_{05}	0.04	2.57	1.58

As a result of conducted research, it was found that yielding capacity of soft spring wheat was on average from 4.13 to 5.54 $t.ha^{-1}$ (LSD_{05}=0.21). The maximum grain yield on average during research period was produced by variety Shirocco -5.54 $t.ha^{-1}$. Varieties Uliublena, Barvysta and Quintus provided grain yield at the level of 4.67, 4.13 and 4.81 $t.ha^{-1}$, respectively (Fig. 2). Modern varieties of spring wheat have a high potential of yielding capacity, but the average yield in recent years under conditions of the forest-steppe of Ukraine was only 2.0–2.5 $t.ha^{-1}$ (Polishuk and Antko, 2020). According to the results of research conducted by Ishchenko (2021), under conditions of unstable moisture, spring wheat produced a yield of 4.5 $t.ha^{-1}$, and the maximum yield of new varieties of soft spring wheat reached 5.85–6.20 $t.ha^{-1}$.

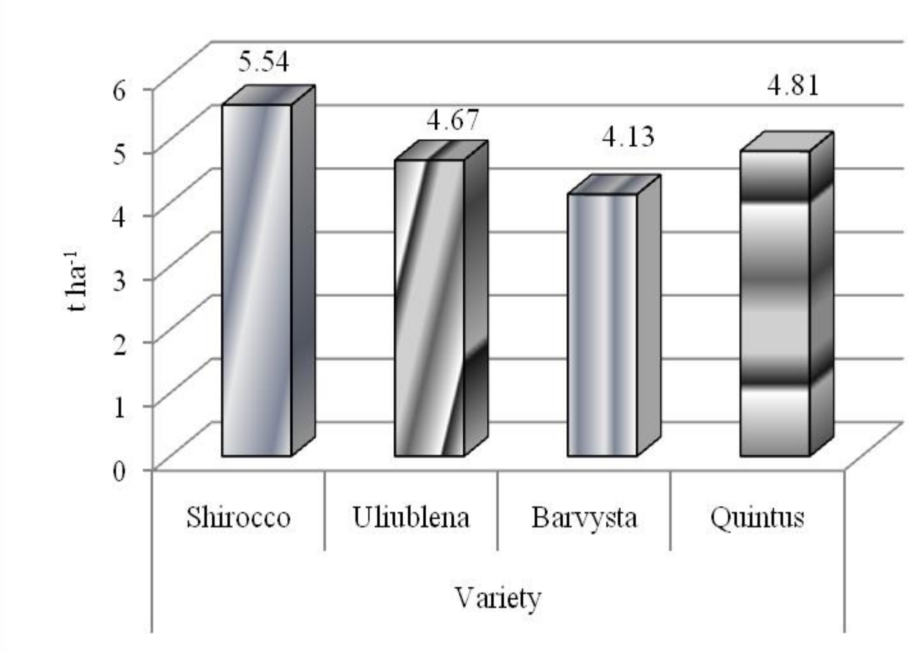

Figure 2. Yielding capacity of soft spring wheat grain depending on varietal characteristics (average for 2020–2022)

It has been experimentally confirmed that grain quality indices of modern varieties of spring wheat depend to a greater extent on varietal characteristics than on weather conditions. Cultivation improvement of new varieties of spring wheat, taking into account economic feasibility of grain production for this crop, is of particular importance (Lozinska and Chevstuk, 2019). Spring wheat grain has high baking and cereal qualities, contains more protein than winter wheat grain. Soft spring wheat grain has a protein content of 14–16% and a gluten content of 28–40% (Hospodarenko, 2001; Andriushchenko, 2002). In the studies conducted by Sumy National Agrarian University, the crude gluten content in the studied varieties ranged from 29.0 to 32.5% (LSD_{05}=0.71). The highest gluten content was obtained in the variant with variety Shirocco –32.5%, and the lowest - variety Barvysta –29.0%. Varieties Uliublena and Quintus showed gluten content at the level of 31.1 and 32.0%, respectively (Table 5).

Table 5. Quality of soft spring wheat grain depending on varietal characteristics (average for 2020–2022)

Variety	Gluten content, %	Protein content, %
Shirocco	32.5	16.3
Uliublena	31.1	15.0
Barvysta	29.0	14.8
Quintus	32.0	15.8
LSD_{05}	0.71	0.46

Protein content of variety Shirocco was 16.3%, variety Uliublena –15.0%, variety Barvysta –14.8%, and variety Quintus –15.8%. Thus, the highest protein content was obtained from variety Shirocco –16.3% (Table 5).

CONCLUSIONS

As a result of the research, it was found that the highest coefficient of productive tillering of spring wheat plants was obtained in the Shirocco variety – 1.30, and the number of productive shoots was 577 pcs m^2. In the experiment, the maximum leaf surface area was noted in the earing phase. When sowing the Shirocco variety, the leaf surface area was the largest and amounted to 43.2 thousand m^2ha^{-1}. On average, over the years of the study, the plants of the Shirocco variety had the highest ear weight –1.51 g, the lowest ear weight was obtained in the Barvysta variety 1.25 g. It was found that the largest number of grains in the ear of the spring wheat plant was in the Quintus variety –31.2 pcs., and the smallest for the Barvysta variety –24.0 pcs. For the Shirocco and Uliublena varieties, the number of grains was at the level of 25.0 and 24.7 pcs.

In the conditions of the natural and climatic zone of the Sumy region (north-eastern Forest-Steppe of Ukraine), it is proposed to grow the Shirocco variety to obtain a yield of spring wheat at the level of 5.54 $t.ha^{-1}$ with a gluten content of 32.5%, protein content of 16.3%.

REFERENCES

Andriushchenko, A. (2002). 4–5 million seeds per hectare is optimal. Proposal, 1: 40–41. [In Ukrainian]

Borysonik, Z. B. (2001). Bright ear crops. Kyiv: Harvest, 176 p. [In Ukrainian]

Demydov, O., Kavunets, V., Siroshtan, A., Hudzenko, V. & Khomenko, S. (2017). Spring wheat needs attention. Proposal, 1: 76–80. [In Ukrainian]

Domaratskiy Y., Bazaliy V., Dobrovol'skiy A., Pichura V. & Kozlova O. (2022). Influence of Eco-Safe Growth-Regulating Substances on the Phytosanitary State of Agrocenoses of Wheat Varieties of Various Types of Development in Non-Irrigated Conditions of the Steppe Zone. Journal of Ecological Engineering, 23(8), 299–308. doi.org/10.12911/22998993/150865

Dospekhov, B. A. (1985). Methods of field experience. Moscow: Kolos, 351 p. [In Russian]

Guedioura I., Rahmoune B., Khezzaren A., Dahoumane A. & Laouar N. (2023). Combined effect of soil practices and chemical treatments on weeds growth, soil features, and yield performance in field wheat crop under Mediterranean climate. Agronomy Research 21 (1), 62–77. doi.org/10.15159/AR.22.079

Hospodarenko, H. M. (2001). Features of fertilizing spring barley with clover seeding. Collection of scientific works of Umansky DAU, 51: 47–56. [In Ukrainian]

Hryhoriv, Y., Butenko, A., Kozak, M., Tatarynova, V., Bondarenko, O., Nozdrina, N., Stavytskyi, A. & Bordun, R. (2022). Structure components and yielding capacity of *Camelina sativa* in Ukraine. Agriculture and Forestry, 68 (3): 93–102. doi:10.17707/AgricultForest.68.3.07

Hrynyk, I. V. (2000). The influence of precursors and the fertilization system on the yield and quality of winter and spring wheat in the conditions of Polissia. Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine, Kyiv. (Candidates's thesis). [In Ukrainian]

Ishchenko, V. A. (2021). Comparative characteristics of productivity of spring wheat varieties in the Northern Steppe of Ukraine. Agrarian innovations, 7: 36–41. doi:10.32848/agrar.innov.2021.7.6

Kalenska, S. M. & Shutyy, O. I. (2015). The formation of structure indexes of durum spring wheat depending on type elements of technology cultivation. Bulletin of the Sumy National Agrarian University, 3 (29): 170–173. [In Ukrainian]

Konovalov, Yu. B. (1999). Formation of productivity of an ear of spring wheat and barley. Moscow: Kolos, 176 p. [In Russian]

Kumakova, V. A. (1988). Biological bases of spring wheat according to intensive technology. Moscow: Rosagropromizdat, 104 p. [In Russian]

Lozinska, T. P. & Chevstuk, A. V. (2019). Yield end quality of soft wheat varieties in the conditions of the experimental field Belotserkiv NAU. Scientific journal ΛΌΓΟΣ, 2: 73–75. [In Ukrainian]

Lozinska, T. P. & Fedoruk, Yu. V. (2017). Biological features of the formation of productivity of spring wheat in the conditions of the forest-steppe of Ukraine. Agriculture, 7(7): 3–8. [In Ukrainian]

Lukianenko, P. P. (1990). Selected writings. Moscow: Agropromizdat, 428 p. [In Russian]

Lykhovyd P. (2023). Choosing Important Traits for the Model of High-Yielding Winter Wheat Variety Based on the Results of Regional Ecological Varietal Testing. Journal of Ecological Engineering, 24(6), 8–12 doi.org/10.12911/22998993/162135

Melnyk, S. I., Sytnyk, V. P., Lazar, T. I., Voitov, I. M. & Kozatskyi, D. V. (2006). Recommendations for growing spring wheat in the forest-steppe of Ukraine. Kharkiv, 23 p. [In Ukrainian]

Nazarenko, M., Semenchenko, O., Izhboldin, O. & Hladkikh, Y. (2021): French winter wheat varieties under ukrainian north steppe condition. Agriculture and Forestry, 67 (2): 89–102. doi:10.17707/AgricultForest.67.2.07

Pidoprygora, V. S. & Pisarenko, P. V. (2003). Workshop on the basics of scientific research in agronomy. Poltava: Intergraphics, 138 p. [In Ukrainian]

Pidruchna, O. V. (2000). The influence of mineral fertilizers on the yield and grain quality of spring durum wheat under irrigation conditions in the south of Ukraine. National Agrarian University, Kyiv. (Candidates’s thesis). [In Ukrainian]

Polishuk, M. I. & Antko, R. A. (2020). Improvement of technological approvals of spring wheat production in the conditions of the right-bank forest Ukraine. Agriculture and forestry, 17: 64–73. doi:10.37128/2707-5826-2020-2-6

Radchenko, M. V., Trotsenko, V. I., Butenko, A. O., Masyk, I. M., Hlupak, Z. I., Pshychenko, O. I., Terokhina, N. O., Rozhko, V. M. & Karpenko, O. Y. (2022). Adaptation of various maize hybrids when grown for biomass. Agronomy Research, 20(2): 404–413. doi:10.15159/AR.22.028

Radchenko, M. V., Trotsenko, V. I., Hlupak, Z. I., Zakharchenko, E. A., Osmachko, O. M., Moisiienko, V. V., Panchyshyn, V. Z., & Stotska, S. V. (2021). Influence of mineral fertilizers on yielding capacity and quality of soft spring wheat grain. Agronomy Research 19 (4): 1901–1913. doi:10.15159/AR.21.104

Shelepov, V. V., Malasai, V. M. & Penzev A. F. (2004). Morphology, biology, economic value of wheat. Mironovka, 524 p. [In Russian]

Shevnikov, D. M. (2012). The influence of mineral fertilizers on the nutrient regime of the soil during the cultivation of durum spring wheat. Bulletin of the Poltava State Agrarian Academy, 2: 203–206. [In Ukrainian]

Sokolov, V. M. (2011). Perspectives of breeding and seed production of grain crops. Scientific reasons for the intensification of grain production in Ukraine, 4: 26–34. [In Ukrainian]

Sviderko, M. S., Bolekhivskyi, V. P., Tymkiv, M. Yu. & Kubyshyn, S. Ya. (2004). Effectiveness of spring wheat cultivation technologies in the Western Forest Steppe. Kyiv: ECMO, 212 p. [In Ukrainian]

Usov, O. S. & Manko, K. M. (2015). Peculiarities of forming the yield of durum spring wheat depending on the predecessor and the main tillage. Collection of scientific papers of the Institute of Bioenergy Crops and Sugar Beet, 23: 70–75. [In Ukrainian]

Verhunov, I. M. (2000). Fundamentals of mathematical modeling for analysis and forecasting of agronomic processes. Kyiv: Noraprint, 146 p. [In Ukrainian]

Vlasenko, V. A. (2006). Assessment of adaptability of soft spring wheat varieties. Varietal research and protection of rights to plant varieties, 4: 93–103. [In Ukrainian]

Xhulaj, Bode, D. & Koto, R. (2022): Estimation of genetic variability of autochthonous wheat (*Triticum aestivum* L.) genotypes using multivariate analysis. Agriculture and Forestry, 68 (1): 131–143. doi:10.17707/AgricultForest.68.1.07

Yula, V. M. & Drozd, M. O. (2020). Productivity of soft spring wheat under adaptive cultivation technologies in the northern Forest Steppe. Collection of scientific papers of the National Scientific Center of the Institute of Agriculture of the Ukrainian Academies of Agrarian Sciences, 1–2: 98–109. [In Ukrainian]

Yula, V. M. (2010). Features of mineral nutrition of spring wheat depending on agrometeorological and agrotechnical factors. Collection of scientific papers of the National Scientific Center of the Institute of Agriculture of the Ukrainian Academies of Agrarian Sciences, 3: 216–227. [In Ukrainian]

Yula, V. M. (2016). The influence of elements of cultivation technology on grain quality of soft spring wheat of the Nedra variety. Collection of scientific works of the Institute of Agriculture of the National Academy of Agrarian Sciences, 3–4: 154–165. [In Ukrainian]

Zhemela, H. P. & Shevnikov, D. M. (2013). Photosynthetic productivity of durum spring wheat crops depending on mineral fertilizers and biological preparations. Bulletin of the Poltava State Agrarian Academy, 3: 36–40. [In Ukrainian]

Holik, O. V. & Kabatsiura, A. A. (2012). Characterization of the source material of spring wheat and spelled according to the ecological plasticity of productivity. Breeding and seed production, 101: 139–149. [In Ukrainian]

Mehić, E., Kazazić, M., Djapo-Lavic, M., Aliman, J. (2023): Influence of rootstock/cultivar combinations on bioactive compounds in sweet cherry fruits. Agriculture and Forestry, 69 (4): 31-40. doi:10.17707/AgricultForest.69.4.03

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INFLUENCE OF ROOTSTOCK/CULTIVAR COMBINATIONS ON BIOACTIVE COMPOUNDS IN SWEET CHERRY FRUITS

SUMMARY

The present study aimed to investigate the influence of different rootstocks (Gisela 6, Pi-Ku 1, and SL 64) on various parameters, including total phenol content, total anthocyanin content, individual phenol content, and antioxidant activity in the fruits of two sweet cherry cultivars, namely Early Lory and Prime Giant. The determination of total phenolic content was carried out using the Folin-Ciocalteu reagent and spectrophotometric method, resulting in a range of 34.77±1.76 to 88.58±8.83 mg GAE/100 g FW, depending on the specific combination of cultivar and rootstock. The concentration of total anthocyanins, determined through the pH-differential method, varied from 1.08±0.07 to 18.62±0.66 mg CGE/100 g FW. Among the different combinations, the highest levels of total phenolic content and total anthocyanin concentration were found in Early Lory cultivar grafted onto Pi-Ku 1 rootstock. Using HPLC analysis, neochlorogenic acid, catechin, chlorogenic acid, *p*-coumaric acid and quercetin-3-*O*-glucoside were detected as individual phenols, exhibiting significant variation among sweet cherry fruits grafted on different rootstocks. The lowest content of the investigated individual polyphenols was observed in Early Lory grafted onto Pi-Ku 1 rootstock. Furthermore, the ferric reducing antioxidant power assay indicated higher antioxidant activity in Early Lory cultivar compared to Prime Giant. A statistically significant correlation was observed between total phenolic content and antioxidant activity (0.978 $p<0.01$), as well as between anthocyanins and antioxidant activity (0.956 $p<0.01$).

Keywords: anthocyanins, antioxidant activity, cultivar, rootstock, total phenol content

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INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a widely popular fruit globally due to its abundant phytonutrients and bioactive compounds, which contribute significantly to its health-promoting properties (Gonçalves *et al.,* 2018). The bioactive compounds in sweet cherries primarily consist of polyphenols, including phenolic acids and flavonoids, which are known for their antioxidant activity (Gonçalves *et al.,* 2004; Usenik *et al*., 2008; González-Gómez *et al.,* 2010; Usenik *et al.,* 2010; Pacifico *et al.,* 2014; Llupa *et al.,* 2022). Phenolic compounds are recognized for their protective effect against oxidative stress (Szajdek and Borowska, 2008), and numerous studies have associated their consumption with various beneficial health effects (Malaguti *et al.,* 2013; Antognoni *et al.,* 2020; Domínguez-Perles *et al.,* 2020). Furthermore, the levels of phenolic compounds in cherry extracts have been directly linked to their antioxidant activity (Tomás-Barberán *et al.,* 2013).

The production of sweet cherries has been steadily increasing worldwide, including in Bosnia and Herzegovina. In 2023, 22 sweet cherry cultivars covered 5,479 hectares, yielding a total of 9,715 tons (FAOSTAT, 2023) in Bosnia and Herzegovina, although the yields remained relatively low. This is mainly attributed to the use of old varieties and generative rootstocks, which possess high vigor, making harvesting difficult and increasing production costs (Drkenda *et al*., 2012). The quality of sweet cherry fruit primarily relies on the variety genotype (Usenik *et al*., 2008; González-Gómez *et al.*, 2010), environmental conditions in the growing area (Tomás-Barberán *et al*., 2013; Skrzyński *et al.,* 2016), maturity stages (Serradilla *et al.,* 2012), and the rootstock genotype onto which the variety is grafted (Scalzo *et al.,* 2005; Tavarini *et al.,* 2011).

The breeding objectives for new cherry varieties include larger fruit size, reduced susceptibility to fruit cracking, self-fertilization, extended ripening season, desirable red color (either light or dark), firmness, sweetness, and taste (Sansavini and Lugli, 2005). At the same time, the quality of fruits and their health effects need to be considered. There has been no detailed research on the effect of rootstock on the content of polyphenols in sweet cherry grown in Herzegovina region. This research was undertaken to evaluate the composition of bioactive compounds of 2 sweet cherry cultivars (Early Lory and Prime Giant) on 3 rootstocks (Gisela 6, Pi Ku-1 and Santa Lucia 64) under climatic conditions in the Herzegovina region.

MATERIAL AND METHODS

The study took place in 2022 at a commercial orchard in Blagaj, located in the Herzegovina region (Figure 1). The average annual temperature in this region is approximately 14.8 °C and the total precipitation is 1,439.3 mm. The orchard was established in 2014, with a planting distance of 4.5×3.6 m. The soil in the orchard is characterized as slightly alkaline clay loam with a sandy texture (pH H2O 8.04; pH KCl 7.35), and it contains 3.78% humus. The soil has good water permeability, adequate aeration, and stable microaggregates. Drip irrigation was employed and all cherry trees were cultivated using standardized agronomic practices for fertilization, irrigation, and pest control.

To determine the optimal harvest time corresponding to the fruit's commercial maturity, the ripening period was assessed based on indicators such as firmness, color, and soluble solids content.

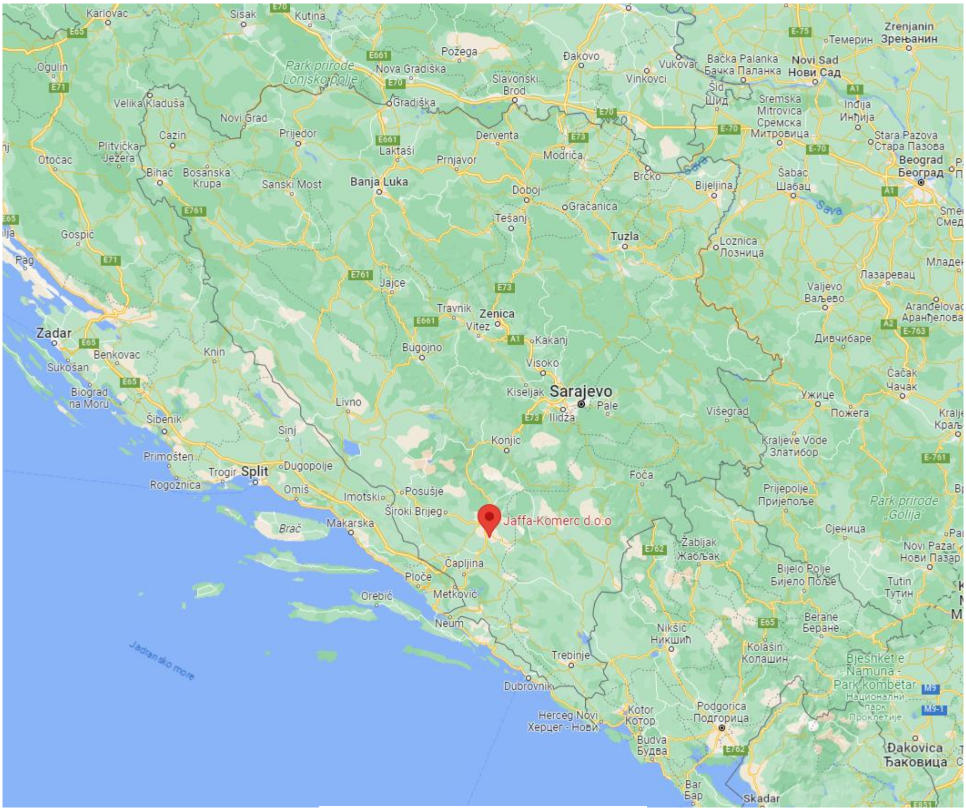

Figure 1. Sampling location

Sample preparation

All fruits were harvested at their full maturity stage and were subsequently utilized for analysis. A blender was used to homogenize 100 g of frozen sweet cherry fruits from each cultivar. From the homogenized sample, 2 g were extracted using 25 mL of 80% acidified methanol (1% HCl) in an ultrasonic bath for 15 minutes at a temperature of 35 °C. The samples were then centrifuged at room temperature for 5 minutes at a speed of 2000 rpm. Subsequently, the samples were ultra-filtrated and stored at -20 °C until analysis.

HPLC analysis

HPLC analysis was performed on Shimadzu HPLC system equipped with a UV/Vis detector as described by Escarpa and González (1999) with some modifications. The chromatographic separation was performed on an Eclipse plus C18 (3.5 µm, 4.6x150 mm) column. The flow rate was 0.8 mL/min, the sample injection was 10 μL. The temperature in the column oven was set to 35 °C. Total run time was 110 min per sample. For separation mobile phase composed of an aqueous solution of phosphoric acid concentration of 0.01 M (mobile phase A) and methanol (mobile phase B) was used with a gradient elution as follows: 18% B, 0-30 min; 30% B, 30-70 min; 45% B, 70-75 min; 100% B, 75-80 min; 18% B, 80-110 min.

Measurements were performed at 320 nm for neochlorogenic acid, catechin and chlorogenic acid, 280 nm for *p*-coumaric acid and 370 nm for quercetin-3-*O*-glucoside. Polyphenolic compounds were identified by comparing retention times with those of authentic standards. Calibration curve of the standards was made by diluting standard mix in acidified 80% methanol to 5-20 μg/mL for standards. Samples were spiked with standard solution for confirmation. All results were expressed as mg per 100 g of fresh weight (mg/100 g FW).

Determination of total phenolic content (TP)

Total phenolic content was determined by Folin-Ciocalteu method as described by Singleton *et al*. (1999) and as previously described by Kazazic *et al*. (2016). Gallic acid was used to prepare the standard curve, and the results were expressed as mg of gallic acid equivalents per 100 g of fruits FW (mg GAE/100 g FW).

Determination of total anthocyanin content (TA)

Total anthocyanin content was determined using the pH-differential method as described by Zhishen *et al.* (1999) with some modifications described by Kazazic *et al*. (2022). The absorbance was measured simultaneously at 510 nm and 700 nm with spectrophotometer after 20 min. The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of fruits FW (mg CGE/100 g FW).

Ferric reducing antioxidant power (FRAP) assay

A modified FRAP method was used to determine antioxidant activity as described in work by Kazazic *et al*. (2016). This method is based on the reduction of the colorless iron (III)-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous form (Fe^{2+}) of intense blue color. The antioxidant activity was determined using the calibration curve and represented as mmol $FeSO_4$ equivalents per 100 g of fruits FW (mmol Fe^{2+}/100 g FW).

Statistical analysis

The results obtained in each analysis were analyzed with Excel (Microsoft Corporation, USA) and IBM SPSS Statistics 25 software (USA). All experiments were conducted in triplicates. The values were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

The sweet cherry is an excellent source of many nutrients and secondary metabolites and can contribute to a healthy diet. Polyphenols are secondary metabolites that are involved in antioxidative defense of plants against biotic and abiotic stresses. Polyphenols are bioactive compounds that play a key role in sweet cherry quality attributes since they contribute to color, taste, aroma and flavor but also have beneficial effects on human health. Influence of rootstock on the content of bioactive compounds in sweet cherry cultivars has been more extensively studied last decade.

Total phenolic content in examined cultivar/rootstock combination varied from 34.77±1.76 to 88.58±8.83 mg GAE/100 g FW (Table 1).

Table 1. The content of total phenols (TP), total anthocyanins (TA) and antioxidant activity (AA) by FRAP method in sweet cherries

Cultivar/rootstock	TP (mg GAE/100 g FW)	TA (mg CGE/100g FW)	AA (mmol Fe^{2+} /100g FW)
E.Lory/Gisela 6	83.73±1.85	17.53±0.88	0.79±0.01
E.Lory/Pi-Ku 1	88.58±8.83	18.62±0.66	0.81±0.00
E.Lory/SL 64	71. 80±7.01	10.35±0.29	0.63±0.02
P.Giant/Gisela 6	41.17±4.36	1.08±0.07	0.30±0.00
P.Giant/Pi-Ku 1	34.77±1.76	2.86±0.07	0.30±0.01
P.Giant/SL 64	42.11±0.01	4.79±0.07	0.35±0.02

The cultivar Early Lory grafted onto Pi-Ku 1 showed the highest level of total phenolic content (88.58±8.83 mg GAE/100 g FW) and highest total anthocyanin content (18.62±0.66 mg CGE/100 g FW).

Total phenolic content of 91.3 mg GAE/100 g in Early Lory cultivar was previously reported by Eroğul (2016) which is in accordance with our results. Legua *et al.* (2017) reported that Prime Giant cultivar had total phenolic content 30 mg GAE/100 g, which is slightly lower than the results reported in this study. Prime Giant grafted onto SL 64 rootstock had total phenolic content of 42.11±0.01 mg GAE/100 g which is lower than 74.84±4.23 mg GAE/100 g reported by Carrión-Antolí *et al.* (2022). Schmitz-Eiberger *et al.* (2012) determined total phenol content of Prime Giant cultivar grafted onto Gisela 5 (35.6±0.8 mg GAE /100 g FW). These differences can be due to the complexity of phenols and various environmental conditions during the growing period of cultivars. Weather conditions during cherry growth may have a profound influence on the total phenolics level. Composition of phenolics and antioxidant activity of the cherry fruit is strongly influenced during ripening stages (Mahmood *et al.,* 2013). The synchronization of harvesting time within a commercial orchard would benefit to growers. The distribution of the s-alleles linked with the flowering and fruit ripening time for cherries cultivars has provided valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time (Dervishi *et al.*, 2022).

Total anthocyanin content varied from 1.08±0.07 to 18.62±0.66 mg CGE/100 g FW in investigated samples. Total anthocyanins of Prime Giant cultivar were in accordance with the results reported by Serrano *et al.* (2009). Prime Giant cultivar showed lower concentration of total anthocyanin content compared to Early Lory cultivar. This can be explained due to the fact that Prime Giant is considered as light-colored cultivar. Díaz-Mula *et al.* (2009) reported direct relationship between color parameters and anthocyanin concentration. The combination of Prime Giant grafted on Gisela 6 showed the lowest concentration of total anthocyanin content (1.08±0.07 mg CGE/100 g FW). Carrión-Antolí *et al*. (2022) analyzed total anthocyanins in Prime Giant grated onto SL 64 rootstock and reported higher concentration compared to the findings presented in this study.

According to the FRAP assay antioxidant activity was higher in Early Lory cultivar compared to Prime Giant with the highest antioxidant activity detected in Early Lory cultivar grafted onto Pi-Ku 1 rootstock (0.81±0.00 mmol Fe^{2+}/100 g FW). Early Lory cultivar also showed the highest antioxidant activity determined by FRAP method in the study by Eroğul (2016), which aligns with the results of this study.

Previous reports found that higher polyphenol content led to higher antioxidant activity.

The correlations between total phenolic content and antioxidant activity (0.978 $p<0.01$) and total anthocyanins and antioxidant activity (0.956 $p< 0.01$) were statistically significant.

A strong genotype, genotype of rootstock and interaction between the cultivar/rootstock influences the phenolic profile of sweet cherry fruits. Usenik *et al.* (2010) reported that there were significant differences between rootstocks in terms of the content of individual polyphenols in sweet cherry cultivars.

Previous studies show that sweet cherries are a good source of polyphenols such as phenolic acids (Kim *et al*., 2005), anthocyanins, flavanols and flavan–3-ols (Gonçalves *et al*., 2004). In the studied sweet cherry fruits the following individual polyphenols were found: neochlorogenic acid, catechin, chlorogenic acid, *p*-coumaric acid and quercetin-3-*O*-glucoside (Table 2).

Table 2. The content of individual polyphenols expressed in mg/100 g FW

Cultivar/rootstock	Neochlorogenic Acid	Catechin	Chlorogenic Acid	*p*-coumaric Acid	Quercetin-3-*O*-glucoside
E.Lory/Gisela 6	B.D.L.	3.55± 0.01[a]	B.D.L.	0.92± 0.01[a]	3.30 ± 0.03 [e,d]
E.Lory/Pi-Ku 1	B.D.L.	N.D.	0.83 ± 0.01[a]	N.D.	3.45 ± 0.01 [f,a,b]
E.Lory/SL 64	B.D.L.	3.11± 0.02[a]	B.D.L.	18.54± 0.07 [d,a]	2.58 ± 0.02[d]
P.Giant/Gisela 6	7.12 ± 0.03 [c,a]	31.66± 0.13 [d,b,c]	N.D.	N.D.	2.27 ± 0.03[c]
P.Giant/Pi-Ku 1	3.51 ± 0.02[a]	13.97± 0.03 [b,c]	B.D.L.	9.63± 0.03 [a,c]	1.93 ± 0.01 [b,c]
P.Giant/SL 64	4.52 ± 0.02[b]	18.33± 0.07[c]	B.D.L.	9.88± 0.02 [a,d]	1.46 ± 0.02 [a,b]

Note: B.D.L. - below detection level; N.D. - not detected. Means in the same columns followed by the same letter (a–f) are not significantly different at the 5% level of probability ($p<0.05$)

The lowest content of the investigated individual polyphenols was measured in Early Lory grafted onto Pi-Ku 1 rootstock. Content of quercetin-3-*O*-glucoside of Early Lory cultivar in combination with rootstocks was decreasing in following order Pi-Ku 1>Gisela 6>SL 64. Content of *p*–coumaric acid was decreasing in Early Lory cultivar in combination with rootstocks in following order SL 64>Gisela 6 but was not detected in Early Lory/Pi-Ku 1. Catechin was not detected in Early Lory/Pi-Ku 1, while higher content was detected in Early Lory/Gisela 6 compared to Early Lory/SL 64. Neochlorogenic acid was below detection level in Early Lory cultivar. Chlorogenic acid was only detected in Early Lory cultivar grafted onto Pi-Ku 1 rootstock.

Content of neochlorogenic acid and catechin of Prime Giant cultivar in combination with rootstock was increasing as following order Pi-Ku 1<SL 64<Gisela 6, whereas for quercetin-3-*O*-glucoside SL 64<Pi-Ku 1<Gisela 6. *p*-coumaric acid was not detected in Prime Giant/Gisela 6 cultivar/rootstock combination but higher concentration was detected in Prime Giant/SL 64 compared to Prime Giant/Pi-Ku 1 combination. Chlorogenic acid was below detection level in Prime Giant cultivar grafted onto Pi-Ku 1 and SL 64 and not detected in Prime Giant/Gisela 6.

Prime Giant cultivar showed higher concentration of catechin and *p*-coumaric acid than reported by Gonçalves *et al.* (2021), while the chlorogenic acid was below detection level.

There are no previous reports available to our knowledge about the concentration of individual polyphenols in Early Lory and Prime Giant cherry cultivars grafted onto Pi-Ku 1, Gisela 6 and SL 64 rootstocks. From this research we can conclude that individual phenols investigated in this study are not predominant polyphenols in these cultivars.

Results showed that the content of individual polyphenols in sweet cherries differed significantly depending on rootstock.

CONCLUSIONS

The results obtained demonstrate that the content of bioactive compounds in the fruits of the examined Early Lory and Prime Giant cultivars is significantly influenced by the rootstocks under investigation (Pi-Ku 1, Gisela 6, and Santa Lucia 64), as well as the interaction between the cultivar and rootstock. Based on the results we can conclude that total phenolic content, total anthocyanins, and antioxidant activity were highest when Early Lory cultivar was grafted onto Pi-Ku 1. On the contrary, when the Prime Giant cultivar was grown on the SL 64 rootstock, it displayed the greatest concentration of the studied bioactive compounds and demonstrated the highest antioxidant activity. Metabolic processes that occur during the joining of scion and rootstock are still not completely understood since it involves complex physiological factors. Therefore, it is important to detect incompatible graft combinations early to prevent financial losses and delays for agricultural producers. At the same time results can be used as recommendation to consumers in terms of their health value and quality of fruits.

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REFERENCES

Antognoni, F., Potente, G., Mandrioli, R., Angeloni, C., Freschi, M., Malaguti, M., Hrelia, S., Lugli, S., Gennari, F., Muzzi, E. & Tartarini, S. (2020). Fruit quality characterization of new sweet cherry cultivars as a good source of bioactive phenolic compounds with antioxidant and neuroprotective potential. Antioxidants, 9(8): 667.

Carrión-Antolí, A., Martínez-Romero, D., Guillén, F., Zapata, P.J., Serrano, M. & Valero, D. (2022). Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. Front.Plant Sci., 13: 1-11.

Dervishi, A., Çakalli, A., Miho, I., Lazaj, A. & Gixhari, B. (2022). Identification of self incompatibility genotypes in sweet cherry commercial cultivars. Agric. For., 68(3): 83-91.

Díaz-Mula, H.M., Castillo, S., Martınez-Romero, D., Valero, D., Zapata, P.J., Guille, F. & Serrano, M. (2009). Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage. Food Sci. Technol.Int., 15(6): 535-543.

Domínguez-Perles, R., Baenas, N. & García-Viguera, C. (2020). New insights in (Poly)phenolic compounds: from dietary sources to health evidence. Foods, 9: 543.

Drkenda, P., Spahić, A., Spahić, A. & Begić-Akagić, A. (2012). Testing of ‘Gisela 5’ and ‘Santa Lucia 64’ cherry rootstocks in Bosnia and Herzegovina. Acta Agric. Slov., 99(2): 129.

Eroğul, D. (2016). İzmir İlinde Yetiştirilen Bazı Önemli Kiraz Çeşitlerinin Fiziksel ve Kimyasal Özelliklerinin Belirlenmesi [Determination of physical and chemical properties of some important cherry cultivars grown in İzmir]. Yuzuncu Yil Univ. J. Agric. Sci., 26(4): 579-585.

Escarpa, A. & González, M.C. (1999). Fast separation of (poly)phenolic compounds from apples and pears by high-performance liquid chromatography with diode-array detection. J. Chromatogr. A, 830(2): 301-309.

Food and Agriculture Organization of the United Nations. FAOSTAT (2023). (available at http://www.fao.org/faostat/en/#data/QC).

Gonçalves, B., Landbo, A.K., Knudsen, D., Silva, A.P., Moutinho-Pereira, J., Rosa, E. & Meyer, A.S. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). J. Agric. Food Chem., 52(3): 523-30.

Gonçalves, A.C., Bento, C., Silva, B., Simões, M. & Silva, L.R. (2018). Nutrients, bioactive compounds and bioactivity: The health benefits of sweet cherries (*Prunus avium* L.). Curr. Res. Nutr. Food Sci., 14: 1–20.

Gonçalves, A.C., Campos, G., Alves, G., Garcia-Viguera, C., Moreno, D.A. & Silva, L.R. (2021). Physical and phytochemical composition of 23 Portuguese sweet cherries as conditioned by variety (or genotype). Food Chem., 335: 127637.

González-Gómez, D., Lozano, M., Fernández-León, M.F., Bernalte, M.J., Ayuso, M.C., & Rodríguez, A.B. (2010). Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). J. Food Compos. Anal., 23: 533-539.

Kazazic, M., Djapo, M., & Ademovic, E. (2016). Antioxidant activity of water extracts of some medicinal plants from Herzegovina region. Int. J. Pure Appl. Biosci., 4(2): 85-90.

Kazazic, M., Mehic, E. & Djapo-Lavic, M. (2022). Phenolic content and bioactivity of two sour cherry cultivars and their products. Glas. Hem. Tehnol. Bosne Herceg., 58: 1-6.

Kim, D.O., Heo, H.J., Kim, Y.J., Yang, H.S. & Lee, C.Y. (2005). Sweet and sour cherries phenolics and their protective effects on neuronal cells. J.Agric. Food Chem., 53: 9921-9927.

Legua, P., Domenech, A., Martínez, J.J., Sánchez-Rodríguez, L., Hernández, F., Carbonell-Barrachina, A.A. & Melgarejo, P. (2017). Bioactive and volatile compounds in sweet cherry cultivars. .Food Nutr. Res., 5(11): 844-851.

Llupa, J., Gašić, U., Brčeski, I., Demertzis, P., Tešević, V. & Topi, D. (2022). LC-MS/MS characterization of phenolic compounds in the quince (Cydonia oblonga Mill.) and sweet cherry (*Prunus avium* L.) fruit juices. Agric. For., 68(2): 193-205.

Mahmood, T., Anwar, F., Bhatti, I.A. & Iqbal, T. (2013). Effect of maturity on proximate composition, phenolics and antioxidant attributes of cherry fruit. Pak. J. Bot., 45: 909-914.

Malaguti, M., Angeloni, C. & Hrelia, S. (2013). Polyphenols in exercise performance and prevention of exercise-induced muscle damage. Oxid.Med. Cell. Longev., 2013: 1–9.

Pacifico, S., Di Maro, A., Petriccione, M., Galasso, S., Piccolella, S., Di Giuseppe, A.M.A., Scortichini, M. & Monaco, P. (2014). Chemical composition, nutritional value and antioxidant properties of autochthonous Prunus avium cultivars from Campania Region. Food Res. Int., 64 (Complete): 188–199.

Sansavini, S. & Lugli, S. (2005). New sweet cherry cultivars developed at the University of Bologna. Acta Hortic., 667: 45-51.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B. & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutr. J., 2: 207-213.

Schmitz-Eiberger, M.A. & Blanke, M.M. (2012). Bioactive components in forced sweet cherry fruit (Prunus avium L.), antioxidative capacity and allergenic potential as dependent on cultivation under cover. LWT – Food Sci. Tech., 46: 388-392.

Serradilla, M.J., Martin, A., Ruiz-Moyano, S., Hernandez, A., Lopez-Corrales, M. & De Cordoba, M.G. (2012). Physicochemical and sensorial characterization of four sweet cherry cultivars grown in Jerte Valley (Spain). Food Chem., 133: 1551–1559.

Serrano, M., Diaz - Mula, H.M., Zapata, P.J., Castillo, S., Guillen, F., Martinez-Romero, D., Valverde, J.M. & Valero, D. (2009). Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. J.Agric. Food Chem., 57: 3240–3246.

Singleton. V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth. Enzymol., 299: 152-178.

Skrzyński, J., Leja, M., Gonkiewicz, A. & Banach, P. (2016). Cultivar effect on the sweet cherry antioxidant and some chemical attributes. Folia Hortic., 28: 95–102.

Szajdek, A. & Borowska, E.J. (2008). Bioactive compounds and health-promoting properties of berry fruits: A review. Plant Foods Hum. Nutr., 63: 147–156.

Tavarini, S., Gil, M.I., Tomas- Barberan, F.A., Buendia, B., Remorini, D., Massai, R., Degl'Innocenti, E. & Guidi, L. (2011). Effects of water stress and rootstocks on fruit phenolic composition and physical/chemical quality in Suncrest peach. Ann. Appl. Biol., 158(2): 226-233.

Tomás-Barberán, F.A., Ruiz, D., Valero, D., Rivera, D., Obón, C., Sánchez-Roca, C. & Gil, M. (2013). Health benefits from pomegranates and stone fruit, including plums, peaches, apricots and cherries. In M. Skinner & D. Hunter, eds. Bioactives in fruit: Health benefits and Functional foods. Wiley, Hoboken, pp 125–167, New Jersey.

Usenik, V., Fabčič, J. & Štampar, F. (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). Food Chem., 107: 185-192.

Usenik, V., Fajt, N., Mikulic Petkovsek, M., Slatnar, A., Stampar, F. & Veberic, R. (2010). Sweet cherry pomological and biochemical characteristics influenced by rootstock. J. Agric. Food Chem., 58 (8): 4928–4933.

Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64 (4): 555-559.

Teka, K., Gessesse, T.A., Welday, Y., Birhane, A., Van Rompaey, A. (2023): Can degraded communal hillside allocation to landless youth improve woody vegetation recovery? A study in the drylands of Ethiopia. Agriculture and Forestry, 69 (4): 41-52. doi:10.17707/AgricultForest.69.4.04

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CAN DEGRADED COMMUNAL HILLSIDE ALLOCATION TO LANDLESS YOUTH IMPROVE WOODY VEGETATION RECOVERY? A STUDY IN THE DRYLANDS OF ETHIOPIA

SUMMARY

Field survey, focus group discussions and key informants’ interview were conducted to collect data on the effects of degraded hillsides allocation to landless youth groups. Data on vegetation recovery, and status of physical soil and water conservation structures were collected from 3 allocated and 3 adjacently non-allocated hillsides. Our findings indicated that hillside allocation improved the length of physical soil and water conservation structures by 58% (from 1310 meters ha^{-1} on communal hillsides to 2067 meters ha^{-1} on allocated hillsides). Hillside allocation to landless youth also improved tree survival rate, number of woody species and species diversity by 25%, 14% and up-to 62%, respectively. It can be concluded that allocation of communal hillsides to landless youth resulted in improved land management and vegetation cover on top of their economic benefits. This implies that the strategy can be taken as a potential option to overcome the challenges of land degradation.

Keywords: improved land management; regeneration; species diversity; soil and water conservation; tree survival rate; youth group

INTRODUCTION

Globally, about 10 to 20% of the dry lands are already degraded, and about 12 million ha is degrading each year (Yirdaw *et al*., 2017). The natural vegetation in communal lands was highly degraded for fuel wood, timber and grazing

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(Gebremedhin *et al*., 2001), which in turn led to loss of biodiversity (Oniki *et al*., 2020). For example, more than 80% of the energy used in developing countries such as Tigray, northern Ethiopia, comes from biomass (Gebremichael and Waters-Bayer, 2007; Shahzad *et al*., 2023). Forest destruction mostly occurred on steep communal hillsides due to the existence of freely available natural resources (Gebremedhin *et al.*, 2003; Berhe and Hoag, 2014).

Several alternative solutions such as hillside afforestation, conservation, privatization, state ownership, imposition and enforcement of use rules and regulations have been implemented in Tigray since the beginning of the1990s to achieve better vegetation cover and contribute to improved livelihoods of local communities (Gebremedhin *et al*., 2001; Gebremedhin *et al*., 2003; Alefew, 2016; Meaza *et al*., 2016; Shimelse *et al*., 2017). In 1997, allocation of degraded and unproductive communal hillsides to individuals has been initiated and used as a policy framework by the government and local communities in Tigray to solve land degradation and the economic problems of landless youth (Gebremedhin *et al*., 2001; Haile *et al*., 2006; Meaza *et al.,* 2016; Shimelse *et al*., 2017). For example, in Atsbi-Womberta district, where this study took place, 34,456 ha of communal hillsides were allocated to 47 youth groups/cooperatives (Gebregergs and Abraha, 2013).

Nevertheless, scaling up of these interventions is challenged by scarcity of well-organized and documented research results that indicate the contributions of allocated hillsides to vegetation recovery as well as performance of implemented soil and water conservation measures. To our knowledge, only limited studies such as Meaza *et al*. (2016), and Gebregergs and Abraha (2013) were conducted on related issues. The 1st study focused on the contributions of land allocation to degraded hillsides' re-vegetation; while the 2nd study dealt with the contributions of land allocation to the livelihood of landless farmers. However, the results of these studies cannot be fully adapted to the entire region for at least four major reasons: i) the studied locations were limited to the mid-land agro-ecology (1500-2300 m above sea level), while the region is characterized by the highland (>2300 m above sea level), mid-land (1500-2300 m above sea level), and lowland (<1500 m above sea level) agro-ecologies; ii) they were old, written based on data collected before 2013, which cannot easily fit to the recent development; iii) impact on other indicators such as the performance of the implemented soil and water conservation structures were not adequately evaluated; iv) they focused on degraded hillsides allocated to landless individual farming households, while many hillsides are also allocated to youth groups/cooperatives.

Hence, this study aimed at establishing up-to-date information on the contribution of landless youth groups' managed hillsides to woody vegetation recovery and performance of soil and water conservation structures in the highland agro-ecology.

MATERIAL AND METHODS

Study site

The study was carried-out in Atsbi-Womberta district, in the highlands of Tigray (elevation > 2300 m above sea level), northern Ethiopia (Figure 1). The total area of the district is about 147,096 ha, of which 34,456 ha communal hillsides are allocated to 47 youth groups/cooperatives. The district is geographically located between 13°33’0” - 14°6’0’’N and 39°39’0’’ - 39°54’0’’E. The study was, specifically, piloted in three communal hillsides namely ‘Tikul-emni at Ruba-felleg village, Enda-anahb at Dibab-akorean village and Adefa at Hayellom village’. The 1st site, Tikul-emni, was allocated to a formal youth group/cooperative (composed of 7 male and 10 female members) called “SEGENAT” in 2016. The 2nd site, Enda-anahb, was allocated to a formal youth group/cooperative (composed of 31 male and 17 female members) known as “SEGISELAM” in 2011. The 3rd site, Adefa, was allocated to an informal youth group (composed of 60 male and 20 female members) in 2000.

Based on the 2019 population projection, the district has a total population of 133,813 or 20,089 households. The climate ranges from cool to warm with an average temperature and rainfall of 18°C and 667.8 mm, respectively. The agro-ecology of the district is classified in to highland/Degua (75% of the total area) and midland/Weina-Degua (25% of the total area) (ILRI, 2004). However, all of the studied sites are found in the highland/Degua agro-ecology, which is related to the high population size in such agro-ecologies that requires puting every possible piece of land in to food and energy production (Gete *et al.* 2006; Gashaw *et al*., 2014). The farming system in the area is also characterized as mixed farming in which livestock rearing (such as cattle, shoat, poultry and bee keeping) and crop production (such as *Hordeum vulgare*/barley, *Triticum aestivum*/wheat, *Vicia faba*/Faba bean and *Eragoristic tef*/Tef) are integral components (ILRI, 2004).

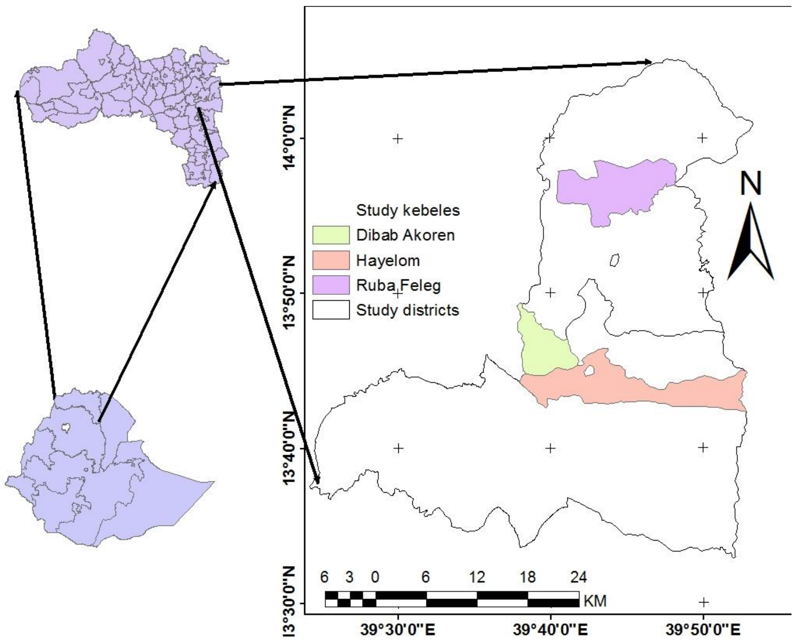

Figure 1. Location of the specific sites in Tigray, Ethiopia

Study method

Data were collected from a total of 74 plots in three allocated and three adjacent communal hillsides (Table 1). The assumption is that both hillsides had similar conditions before land allocation. Field survey (observation and measurement), focus group discussions and key informants' interview were the major primary data collection methods employed. Field survey, with the aim of assessing woody species recovery and existing soil and water conservation measures, was conducted in three parallel transect lines in each study site. Sample plots were laid along each transect at a distance of 100 meters between lines and 40 meters between plots as suggested in Abiyu *et al*. (2011) and Kuma and Shibru (2015).

Table 1. Sample plot size and their distribution (Source: Field survey, 2020).

Study site	Allocated hillsides		Non-allocated hillsides		Total	
	Ha	Plots	Ha	Plots	Ha	Plots
Tikul-emni	2.68	7	2.68	7	5.36	14
Enda-anahb	6.37	13	6.37	13	12.74	26
Adefa	9.18	17	9.18	17	18.36	34
Total	18.23	37	18.23	37	36.46	74

Each sample plot had a square shape (20 m × 20 m) and three nested compartments of different sizes as proposed in Yami *et al*. (2006) and Kuma and Shibru (2015). The woody vegetation recovery status of both land uses was determined through measurement of species composition, density, diversity, and tree survival rate following recommendations in Mengist *et al*. (2005) and Tewolde-Berhan *et al.* (2016). On the 1st compartment (20 m × 20 m plot size), three tasks were accomplished: i) the length, type and quality of the existing physical soil and water conservation structures were recorded as suggested in Walie and Fisseha (2016); ii) species composition, diversity, density and tree survival of planted and naturally grown woody species having ≥ 10 cm diameter at breadth height (dbh), and greater than two-meters in height were recorded as proposed in Yami *et al.* (2006); iii) for species having less than one-meter height, only their number was recorded as put forward in Birhane *et al.* (2006). On the 2nd compartment (5 m × 5 m plot size), both planted and naturally grown sapling trees with $2<\text{dbh}\leq 10$ cm were counted and recorded as suggested in Tewolde-Berhan *et al.* (2016); shrubs with ≥ 2 cm diameter at 30 cm stem height above the soil surface was measured and counted as proposed in Mengist *et al*. (2005) and Yami *et al.* (2006). On the 3rd compartment (2 m × 2 m plot size), the number of naturally regenerated woody species having less than one-meter height was counted as recommended in Mengist *et al*. (2005) and Tewolde-Berhan *et al.* (2016); their survival rate was estimated following equation 1. Woody vegetation status in each site was described in terms of species diversity, species evenness and Simpson's diversity index (Kent, 2011).

$$Survival\ rate\ (\%) = \frac{Number\ of\ seedling\ living}{Total\ number\ of\ seedlings\ planted} \times 100 \quad \text{(Eq.1)}$$

To obtain more reliable information, the field survey data were supported by focus group discussions (FGDs). Therefore, a total of three FGDs each composed of twelve respondents (six from user and six from non-user group) were conducted. Finally, quantitative data obtained through field survey were analyzed by using SPSS. Descriptive statistics such as mean, percentage and frequency were used to present the qualitative results. Independent t-test was employed to analyze the differences between the two land use types (allocated and communal hillsides) and for each specific objective. The implemented soil and water conservation structures, survival rate, diameter at breast height (dbh), and vegetation density were treated as responsible variables, while land use as a group (Asmare and Gure, 2019).

RESULTS AND DISCUSSION

Impact on quality of the implemented physical SWC measures

Major physical soil and water conservation (SWC) measures implemented in the studied sites were hillside terrace, and hillside terrace+trench (Table 2). The results showed that the length of the implemented physical SWC structures was much better on the allocated hillsides, which was 58% higher as compared to the communal hillsides (from 1310 meters ha^{-1} on communal to 2067 meters ha^{-1} on allocated hillsides). Hence, hillside allocation gave a chance for additional hillside terraces and trenches construction on ten of the 37 plots.

Table 2. Occurrence of physical SWC structures in the study sites (Source: Field survey, 2020).

SWC Structure	Tikul-emni		Enda-anahb		Adefa		Total	
	AHS	NAHS	AHS	NAHS	AHS	NAHS	AHS	NAHS
Hs trace m ha^{-1}	16,500	8,500	7,500	20,500	33,000	19,500	57,000	48,500
Hs+trenc m ha^{-1}	0	0	18,000	0	1,500	0	19,500	0
Coverage m ha^{-1}	16,500	8,500	25,500	20,500	34,500	19,500	76,500	48,500
Mean m ha^{-1}	2,357	1,214	1,961	1,576	2,029	1,147	2067	1310
Std.dev	244	447	431	187	514	606	459	593
M.difference	1,143		384		882		123	
T-value	3.22		2.95		4.58		6.14	
p-value	0.015		0.009		0.000		0.000	

AHS=Allocated, NAHS=Non-Allocated

Our findings also indicated that the quality of physical SWC structures on allocated hillsides was more superior to those on the communal hillsides (Table 3). The dimension (height and width) of the implemented SWC structures on allocated hillsides were statistically different (p<0.001) as compared to the communal hillsides. Out of the 37 plots studied on the communal hillsides, physical SWC measures implemented in four plots were totally destructed; while these structures were fully maintained on the allocated ones.

Table 3. SWC structure dimension (width and height) in the study sites (Source: Filed survey, 2020).

Dimension	Tikul-emni		Enda-anahb		Adefa		Total	
	AHS	NAHS	AHS	NAHS	AHS	NAHS	AHS	NAHS
Width								
Mean	0.3	0.27	1.0	0.32	0.32	0.24	0.56	0.28
Std.Dev	0	0.21	0	0.04	0.07	0.14	0.33	0.13
Mean diff	0.04		0.68		0.08		0.28	
T-value	0.367		55.66		2.10		7.32	
p-value	0.73		0.000		0.047		0.000	
Height								
Mean	0.5	0.36	1.18	0.38	0.58	0.30	0.78	0.34
Std.Dev	0.21	0.28	0.06	0.13	0.10	0.17	0.31	0.18
Mean diff	0.14		0.08		0.28		0.44	
T-value	1.34		20.66		5.79		7.32	
p-value	0.205		0.000		0.000		0.000	

AHS=Allocated hillside; NAHS=non-allocated hillside

Impact on vegetation recovery

Tree survival rate

The vegetation regeneration status of woody plants was also evaluated taking tree survival rate as an indicator. Our findings indicated that hillside allocation increased tree survival rate by more than 25% as compared to the communal ones (Table 4).

Table 4. Summary of planted trees and their survival rate (Source: Field survey, 2020)

	Tikul-emni		Enda-anahb		Adefa		Total	
	AHS	NAHS	AHS	NAHS	AHS	NAHS	AHS	NAHS
Planted (N ha^{-1})	2,100	2,010	1,800	1,900	3,900	3,910	7,800	7,820
Counted (N ha^{-1})	1,655	1,000	1,150	820	2,820	1,835	5,616	3,655
Survived (%)	78.8	50.05	64.05	43.2	72.3	46.9	72	46.7
p-value	0.006		0.010		0.009		0.001	

AHS=Allocated hillside; NAHS=Non-allocated hillside

Woody species density

Field survey results indicated a significant difference on the number of woody plant species between the two land use types (Figure 2). Woody species in the order of occurrence on the allocated hillsides were *Acacia seyal* (25.6%) > *Olea africana* (21.3%) > *Dodonea angustifolia* (17%) > *Juniperus procera* (10.9%) > *Rhus vulgaris* (8.1%) > *Acacia etbaica* (9%). Whereas, *Dodonea angustifolia* (18%) > *Calpurnia aurea* (3.3%) > *Carissa edulis* (3.8%) dominated the communal hillsides. Land allocation to youth groups increased the number of trees in a hectare from 25 to 148 trees, a 4.9-fold increase.

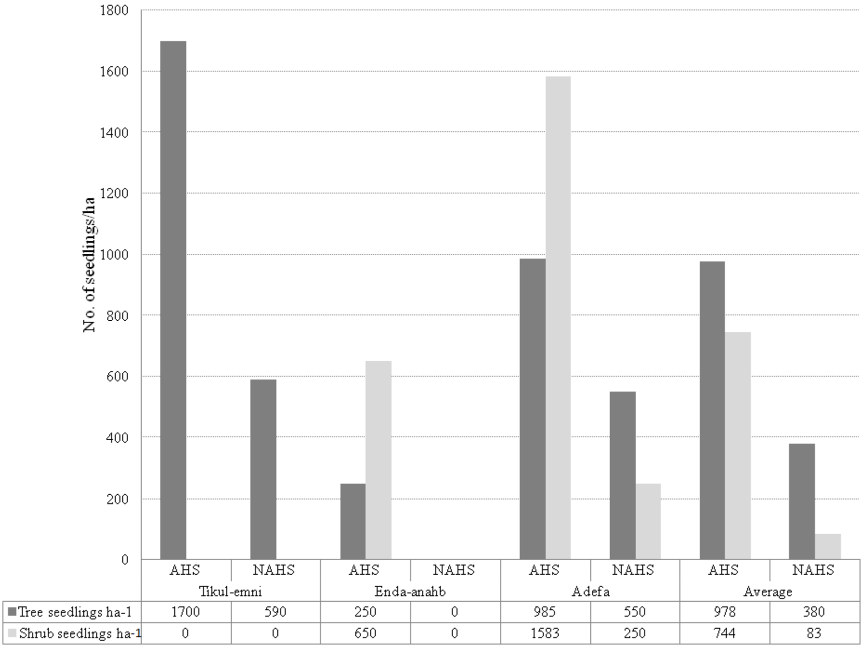

Figure 2. Number of tree and shrub seedlings in a hectare in the study sites

Woody species composition

The experimental results indicated that allocated hillsides had higher species composition than the communal ones (Table 5). Allocated hillsides had 16 woody species representing 12 families; while only 14 species representing 10 families were recorded in the communal hillsides. This implies hillside allocation to landless youth improved woody species and family's composition by 14.3% and 20%, respectively; while species diversity increased by up to 62%.

Table 5. Shannon–Wiener diversity index of species in the study sites (Source: Field survey, 2020)

Species	Tikul-emni		Enda-anahb		Adefa		Total	
	AHS	NAHS	AHS	NAHS	AHS	NAHS	AHS	NAHS
Sh.diversity	1.64	1.10	1.22	0.95	1.78	1.07	2.4	1.48
S. diversity	0.79	0.59	0.68	0.52	0.73	0.59	0.85	0.72
Evenness	0.85	0.69	0.87	0.68	0.68	0.49	0.76	0.56
Abundance	90	164	422	567	541	498	1053	1229

AHS=Allocated hillside; NAHS=non-allocated hillside

Woody species diversity

The Shannon diversity index of woody species indicated that *Eucalyptus globulus, E. camadulensis*, *Euclea schimperi*, *Rumex nervosus* and *Becium grandiflorum* were uniformly distributed all over the sampled plots in the allocated hillsides. However, two of these species (*Calpurnia aurea* and *Carissa edulis*) were absent in the adjacent communal hillsides. *Rumex nervosus* (0.39), *Becium grandiflorum* (0.36) and *Euclea schimperi* (0.35) were more diverse than the other species found in the non-allocated hillsides.

Species evenness

Species equitability (evenness) ranges between 0.33 and 0.75 for non-allocated hillside, while it was 1.0 for the allocated ones. These values indicate that allocated hillsides were more diverse than their adjacent communal hillsides. Similarity assessment results, above 0.58 in most of the studied plots, indicate the species were nearly similar.

Stand basal area, important value index (IVI) and dominancy

Table 6 shows the measurement results of stand basal area, important value index (IVI) and dominancy of both land uses. The survey results indicated higher basal area (3.73 m^2 ha^{-1}) on the allocated than that of communal hillside (0.78 m^2 ha^{-1}). Taking tree basal area as an example, allocated hillsides had higher tree basal area (5 m^2 ha^{-1}) than communal hillsides (0.3 m^2 ha^{-1}). Important value index (IVI) and dominancy values also shown variation between the studied land use types. On the allocated hillsides, *Eucalyptus* species had the highest important value index (IVI); while, *Calpurnia aurea* and *Withania somnifera* had the lowest IVI. Shrubs such as *Euclea schimperi*, *Becium grandiflorum*, and *Rumex nervosus* had the highest IVI in the communal hillsides. The highest IVI indicates that the species is uniformly dispersed with big value of dominancy position.

Table 6. Stand basal area measurement results (Source: Field survey, 2020)

Species	Tikul-emni		Enda-abahb		Adefa		Total	
	AHS	NAHS	AHS	NAHS	AHS	NAHS	AHS	NAHS
Tree g ha^{-1}	1.37	0	8.29	0	6.60	0.20	5.11	0.33
Sapling g ha^{-1}	2.54	0.61	3.00	0	3.21	1.24	2.69	0.94
Shrub g ha^{-1}	0.98	1.21	1.66	1.40	3.41	5.56	2.58	3.14
Total	2.48	0.57	4.50	0.47	3.27	0.74	3.73	0.78
Mean difference						2.54		2.95
T-value						1.75		1.40
p-value						0.009		0.000

AHS=Allocated hillside; NAHS=non-allocated hillside

DISCUSSSION

Quality of the implemented physical SWC measures

The dominance of hillside terrace and hillside terrace+trench in the study area (Table 2) supports the findings of Asnake and Elias (2017) for the hilly and mountainous areas of Ethiopia. Moreover, Desta *et al.* (2005) stated that the dominant presence of hillside terraces on such mountainous areas was related to its suitability to construct on arid and semi-arid environmental conditions. The superior quality of physical SWC structures on allocated hillsides (Table 3) was related to the continuous and improved construction and maintenance of SWC structures by the youth groups that in turn led to the lower destruction and exploitation status of these and other resources in these hillsides (Mekonnen and Tesfahunegn, 2011).

Vegetation recovery

The highest tree survival rate result, 25% higher than the communal ones, shown in Table 4 corresponds with the findings of Jagger *et al.* (2005) that reported community-managed woodlots had lower tree survival rate (approximately 45%) compared to the household managed woodlots with 65% survival rate, an increase by 20%. Limited sense of ownership of the community in the community managed hillsides was the major reason for the poor tree survival rate. Results from the focal group discussions (FGDs) and key informants interview indicated that majority of the local community assume communal hillsides as the property of the local authority. As a result, farmers throw–out seedlings to the surrounding, plant upside down during plantation campaign, and send livestock to graze/ browse in the dark. These reports are in par with the findings of Meaza *et al*. (2016) that pointed out moisture stress and free-ranging by livestock were the major reasons for the poor tree survival rate in the communal hillsides.

In addition to its positive impact on tree survival rate, land allocation to youth groups resulted to a 4.9-fold increase in the number of trees in a hectare (Figure 2). This implies that land allocation to youth groups results in better vegetation recovery as compared to their corresponding communal hillsides. These results are also in accordance to the findings reported in Gebregergs and Abraha (2013) that revealed an increase of tree density in managed fields by a factor of 4.1 over disturbed hillsides. Mengist *et al.* (2005), Mekuria and Aynekulu (2011) and Manaye *et al.* (2019) also reported a similar result that revealed better regeneration potential in managed fields than adjacent communal fields.

Survey results on species diversity have also shown a 33% increase on allocated land as compared to the communal fields (Table 5), which is in par with the results of Asmare and Gure (2019). However, in most of the studied plots, similar plant species were found. This is in agreement with the findings of Manaye *et al*. (2019) that revealed a high species similarity due to the presence of similar edaphic, climatic condition and altitudinal ranges of the existing land use and vegetation types in the past that in turn leads to re-appearing of similar vegetation types.

The survey results, further, indicated a 378% higher basal area on allocated hillsides than those of communal ones, in which *Eucalyptus* species had the highest important value index (IVI) and dominancy. The highest IVI of *Eucalyptus* species in the allocated hillsides was related to its high preference by the community for multiple uses, economic returns, and resistance to water stress and ecological agents (Saadaoui *et al.,* 2017; Birhanu and Kumsa, 2018; Getnet *et al*., 2022). The dominancy of *Euclea schimperi, Becium grandiflorum,* and

Rumex nervosus in the communal hillsides, was also related to their advantage of primary succession and less palatability for browsers (Birhane *et al*., 2007).

CONCLUSIONS

This study assessed the effect of allocated degraded hillsides on woody vegetation recovery and quality of the implemented physical soil and water conservation (SWC) structures by comparing them with adjacent communal hillsides. Survey results showed that the quality and quantity of the implemented SWC measures were much better on the allocated hillsides. Hillside terrace + trench was particularly implemented on the allocated hillsides to boost the growth of planted trees through minimizing soil moisture stress. From the length point of view, the area coverage of SWC was 58% higher on allocated hillsides. Trees survival rate was also 25% higher on allocated hillsides. Moreover, allocated hillsides were better in species composition, diversity and density. Thus, it can be concluded that allocating degraded hillsides to landless youth groups improves woody vegetation recovery, and quality of soil and water conservation structures in addition to their economic benefits.

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REFERENCES

Abiyu A., Lemenih M., Gratzer G., Aerts R., Teketay D., Glatze G., 2011. Status of Native Woody Species Diversity and Soil Characteristics in an Exclosure and in Plantations of Eucalyptus globulus and *Cupressus lusitanica* in Northern Ethiopia. Mountain Research and Development (MRD), 31(2): 144-152. https://doi.org/10.1659/MRD-JOURNAL-D-10-00116.1.

Alefew T., 2016. Assessment on Effectiveness and Sustainability of PSNP Community Based Participatory Reforestation and Hill Land Management through Watershed Management Activity in Dega, Woina Dega and Kolla Agro-ecological Zones: The Case of Amhara Sayint Woreda: South. College of Education and Behavioral Studies Department of Geography and Environmental Education Summer Program. Addis Ababa University, Addis Ababa. Pp. 110.

Asmare M.T., Gure A., 2019. Effect of exclosure on woody species diversity and population structure in comparison with adjacent open grazing land: the case of Jabi Tehnan district north western Ethiopia. Ecosystem Health and Sustainability, 5(1): 98–109. DOI: 10.1080/20964129.2019.1593794.

Asnake B., Elias E., 2017. Challenges and extents of Soil and Water Conservation measures in Guba-Lafto Woreda of North Wollo. Journal of Agricultural Research and Development, 7(2): 103–110. DOI: http://dx.doi.org/10.18685/EJARD(7)2_EJARD-16-012.

Berhe M., Hoag D., 2014. The livelihood effects of landless people through communal hillside conservation in Tigray Region, Ethiopia. Journal of Development and Agricultural Economics, 6(7): 309–317. DOI:10.5897/JDAE2014.0571.

Birhane E., Teketay D., Barklund P., 2006. Actual and potential contribution of exclosures to enhance biodiversity of woody species in the drylands of Eastern Tigray. Journal of the Drylands, 1(2): 134-147. ISSN 1817-3322.

Birhane E., Teketay D., Barklund P., 2007. Enclosures to Enhance Woody Species Diversity in The Dry Lands of Eastern Tigray, Ethiopia. East African Journal of Sciences, 1 (2) 136-147. DOI: 10.4314/eajsci.v1i2.40352.

Birhanu S., Kumsa F., 2018. Review on Expansion of Eucalyptus, its Economic Value and Related Environmental Issues in Ethiopia. International Journal of Research in Environmental Science, 4(3): 41-46. DOI: http://dx.doi.org/10.20431/2454-9444.0403005.

Desta L., Carucci V., Wendem-Ageňehu A., Abebe Y., 2005. Community Based Participatory Watershed Development: A Guideline. Ministry of Agriculture and Rural Development, Addis Ababa, Ethiopia. Pp. 74.

Gashaw T., Behaylu A., Tilahun A., Fentahun T., 2014. Population Growth Nexus Land Degradation in Ethiopia. Journal of Environment and Earth Science 4 (11): 54 - 57. ISSN 2225-0948

Gebregergs H.M., Abraha G.G., 2013. Re-Vegetation of Degraded Hillsides Through Household Tree Planting in Northern Highlands of Ethiopia. International Journal of Scientific Research, 2(3): 2277 – 8179. DOI:10.15373/22778179/MAR2013/59.

Gebremedhin B., Pender J., Ehui S.K., Haile M., 2003. Policies for sustainable land management in the highlands of Tigray, northern Ethiopia: Summary of papers and proceedings of a workshop held at Axum Hotel, Mekelle, Ethiopia, 28–29 March 2002. Socio-economic and Policy Research Working Paper 54. ILRI (International Livestock Research Institute), Nairobi, Kenya. 75 pp.

Gebremedhin B., Penderb J., Tesfay G., 2001. Community Resource Management: The Case of Grazing Lands in Northern Ethiopia" (2001). International Conference on African Development Archives. 49. Pp. 18.

Gebremichael Y., Waters-Bayer A., 2007. Integrating environment and local development in Tigray Region of Ethiopia. Irish Aid. Pp. 32. ISBN: 9781843696612. Available at: http://pubs.iied.org/pubs/pdfs/12539IIED.pdf.

Gete Z., Menale K., Pender J., Mahmud Y., 2006. Stakeholder analysis for Sustainable Land Management (SLM) in Ethiopia: Assessment of opportunities, strategic constraints, information needs, and knowledge gaps. Environmental Economics Policy Forum for Ethiopia (EEPFE). Ethiopia. Pp. 97.

Getnet M.T., Ketema M., Alemu B., Demilew G., 2022. An Assessment on Socio-Economic Impacts of Smallholder Eucalyptus Tree Plantation in the case of Northwest Ethiopia. European Online Journal of Natural and Social Sciences, 11 (1): 250-262. SSN 1805-3602.

Haile M ., Herweg K., Stillhardt B., 2006. Sustainable Land Management – A New Approach to Soil and Water Conservation in Ethiopia. Geography. DOI:10.7892/BORIS.19217.

ILRI. 2004. Atsbi Wemberta Pilot Learning Site diagnosis, program design and atlas. Nairobi, Kenya: ILRI.Pp. 75.

Jagger P., Pender J., Gebremedhin B., 2005. Trading Off Environmental Sustainability for Empowerment and Income: Woodlot Devolution in Northern Ethiopia. World Dev., 33: 1491–1510

Kent M., 2011. Vegetation Description and Analysis: A practical approach (2nd Edition). Wiley-Blackwell. Pp. 448. ISBN: 978-1-119-96239-7.

Kuma M., Shibru S., 2015. Floristic Composition, Vegetation Structure, and Regeneration Status of Woody Plant Species of Oda Forest of Humbo Carbon Project, Wolaita, Ethiopia. Journal of Botany, 2015(2):1-9. DOI:10.1155/2015/963816.

Manaye A., Negash M., Alebachew M., 2019. Effect of degraded land rehabilitation on carbon stocks and biodiversity in semi-arid region of Northern Ethiopia. Forest Science and Technology, 1–9. https://doi.org/10.1080/21580103.2019.1592787.

Meaza, H., Tsegaye D., Nyssen J., 2016. Allocation of degraded hillsides to landless farmers and improved livelihoods in Tigray, Ethiopia, Norsk Geografisk Tidsskrift - Norwegian Journal of Geography, 70(1): 1-12. https://doi.org/10.1080/00291951.2015.1091033.

Mekonnen K., Tesfahunegn G.B., 2011. Impact assessment of soil and water conservation measures at Medego watershed in Tigray, northern Ethiopia. Maejo International Journal of Science and Technlogy, 5(03): 312-330. ISSN 1905-7873.

Mekuria W., Aynekulu E., 2011. Exclosure Land Management for Restoration of the Soils in Degraded Communal Grazing Lands in Northern Ethiopia. Land Degradation & Development, 11. https://doi.org/10.1002/ldr.1146.

Mengist T., Teketay D., Hulten H., Yemshaw Y., 2005. The role of enclosures in the recovery of woody vegetation in degraded dryland hillsides of central and northern Ethiopia'. Journal of Arid Environments, 60(2): 259–281. https://doi.org/10.1016/j.jaridenv.2004.03.014.

Oniki S., Berhe M., Takenaka K., 2020. Efficiency Impact of the Communal Land Distribution Program in Northern Ethiopia. Sustainability 12, 4436. DOI:10.3390/su12114436.

Saadaoui, E., Ben Yahia, K., Dhahri, S., Ben Jamaa, M.L., Khouja, M.L., 2017. An overview of adaptative responses to drought stress in Eucalyptus Spp. Forestry Studies | Metsanduslikud Uurimused 67, 86–96. DOI:10.1515/fsmu-2017-0014.

Shahzad U., Elheddad M., Swart J., Ghosh S., Dogan B., 2023. The role of biomass energy consumption and economic complexity on environmental sustainability in G7 economies. Business Strategy and the Environment, 32(1): 781–801. https://doi.org/10.1002/bse.3175.

Shimelse S., Bekele T., Nemomissa S., 2017. Determinants of sustainability of exclosure establishment and management practices in Tigray Region, Ethiopia. International Journal of Scientific and Research Publications, 7(12): 442–456. ISSN 2250-3153.

Tewolde-Berhan S., Ralph M., Muys B., Haile M., 2016. Vegetation Improvement in Communal Closed Areas in Tigray, Ethiopia. Tfap. Pp. 1–8.

Walie S.D., Fisseha G., 2016. Evaluation of Land Use Types and Physical Soil and Water Conservation Structures in Wyebla Watershed, Northwest Ethiopia. International Journal of Environmental Protection, 6(1): 90–96. DOI:10.5963/IJEP0601008.

Yami M., Gebrehiewet K., Stein M., Mekuria W., 2006. Impact of area enclosures on density, diversity, and population structure of woody species: the Case of May Ba'ati-Douga Tembien, Tigray. Pp. 99-116. Available at: https://www.researchgate.net/publication/225187736.

Yirdaw E., Mulualem T., Monge A. 2017. Rehabilitation of degraded dryland ecosystems – review'. Silva Fennica. 51(1):1–32. https://doi.org/10.14214/sf.1673.

Karpets, Y.V., Taraban, D.A., Kokorev, A.I., Yastreb, T.O., Kobyzeva, L.N., Kolupaev, Y.E. (2023): Response of wheat seedlings with different drought tolerance to melatonin action under osmotic stress. Agriculture and Forestry, 69 (5): 53-69. doi:10.17707/AgricultForest.69.4.05

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RESPONSE OF WHEAT SEEDLINGS WITH DIFFERENT DROUGHT TOLERANCE TO MELATONIN ACTION UNDER OSMOTIC STRESS

SUMMARY

Melatonin is a multifunctional molecule whose biological activity has been intensively studied in recent years in the context of plant adaptation to abiotic stresses, including drought. However, comprehensive studies on the effect of melatonin on the status of antioxidant and osmoprotective systems in wheat varieties with different drought adaptation strategies have been lacking. We studied the effect of melatonin on the growth and functioning of stress protection systems of wheat (*Triticum aestivum* L.) seedlings of Tobak (drought-tolerant) and Doskonala (drought-sensitive) varieties. Two-day-old etiolated seedlings were transferred to a 15% PEG 6000 solution for 2 days to create a model drought. The addition of melatonin to the incubation medium of seedlings at concentrations in the range of 0.2-5.0 μM significantly alleviated the inhibition of root and shoot growth of seedlings caused by drought. The effects of melatonin were more pronounced in the drought-sensitive cultivar Doskonala. Melatonin treatment almost completely eliminated the effect of increased hydrogen peroxide content under drought conditions in both varieties. Also in Doskonala variety, melatonin treatment reduced stress-induced accumulation of lipid peroxidation products. Drought caused a decrease in superoxide dismutase activity in both varieties, while melatonin treatment helped to maintain the enzyme activity at a level close to that of the control variants. In both varieties, melatonin treatment also reversed the stress-induced decrease in guaiacol peroxidase activity. Under

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the influence of melatonin, proline accumulation was significantly enhanced in Doskonala under drought conditions. Drought caused a decrease in sugars and flavonoid compounds, while melatonin mitigated these effects in both varieties. It is concluded that melatonin significantly modulates the adaptive responses of etiolated wheat seedlings to drought and that its effects depend on cultivar characteristics.

Keywords: *Triticum aestivum*, melatonin, drought tolerance, reactive oxygen species, antioxidant system, proline, soluble carbohydrates, flavonoids

INTRODUCTION

A global analysis of published results from studies conducted over the past four decades has shown that wheat yield losses due to water stress are at least 20% (Daryanto *et al.*, 2016). Drought stress causes a number of biochemical, morphophysiological, and anatomical disturbances in plants that occur at all stages of plant development, from seed germination to the final stages of the generative phase (Cui *et al*., 2018; Tiwari *et al.*, 2021). Drought tolerance in plants is the result of numerous interrelated mechanisms that operate at the molecular, cellular, and organismal levels. Among them, changes in the state of the stomatal apparatus, accumulation of various osmolytes in cells, activation of aquaporins, increased functioning of the antioxidant system are currently considered to be the key ones (Nawaz *et al*., 2019; Pržulj *et al*., 2020; Singh *et al.*, 2021; Vignesh, Palanisamy 2021; Kolupaev *et al.*, 2023a). The regulation of these processes involves numerous groups of biomolecules that exhibit signaling and hormonal activity (Yemets *et al*., 2019; Kosakivska *et al*., 2022).

In addition to the classical "drought hormone" abscisic acid (ABA) (Jogawat *et al*., 2021), the role of other groups of hormones—salicylic acid (Singh *et al*., 2021; Rouhnezhad *et al.*, 2023), jasmonates (Kolupaev *et al*., 2023b), and brassinosteroids (Ribeiro *et al*., 2019) - in drought adaptation has been intensively studied over the past two decades. Also in recent years, knowledge of the plant functions of neurotransmitters, which were previously discovered and studied in mammals, has been intensively accumulated. The main of these compounds are acetylcholine, biogenic amines (dopamine, noradrenaline, adrenaline, histamine), and indolamines (melatonin and serotonin) (Akula, Mukherjee, 2020). Melatonin (N-acetyl-5-methoxytryptamine) is now recognized as a pleiotropic signaling molecule that plays an important role in regulating responses to various stresses (Buttar *et al.,* 2020). It was first discovered in plants only in 1995 (Dubbels *et al*., 1995). In recent years, however, melatonin has become a popular topic in plant biology (Ayyaz *et al*., 2022).

A number of studies conducted on plants of different species have found an increase in endogenous melatonin levels in response to stressors (Buttar *et al*., 2020), including the effects of drought (Arnao, Hernández-Ruiz, 2013). A large body of evidence has also been accumulated on the positive effects of exogenous melatonin on plant tolerance to abiotic stressors (Ciu *et al*., 2018; Li *et al.*, 2020). However, the phenomenology rather than the mechanisms of melatonin's stress-protective effects have been predominantly studied. At least one reason for the protective effects of melatonin is related to the modification of redox homeostasis. In particular, direct antioxidant effects of melatonin have been hypothesized, given its ability to interact with ROS and its presence in significant amounts in plants (Arnao, Hernández-Ruiz, 2015; Fan *et al*., 2018; Ahamad *et al.,* 2021). It has been reported that one molecule of melatonin can bind up to 10 free radicals, which may be higher than the efficiency of superoxide dismutase (SOD) action (Ye *et al*., 2016). However, the effects of melatonin on redox homeostasis are not limited to direct antioxidant effects. Many studies have reported the induction of antioxidant enzyme gene expression by melatonin (Martinez *et al*., 2018; Sun *et al.*, 2018; Zhao *et al*., 2018) and its effect on the synthesis of low-molecular-weight antioxidants (Ahmad *et al*., 2019; Awan *et al*., 2023).

A hallmark of the plant response to drought stress is the accumulation of various osmolytes, mainly proline (Joseph *et al.*, 2015) and sugars (Mukarram *et al.*, 2021), as well as a number of secondary metabolites (Ma *et al*., 2014). All of these compounds have antioxidant activity and may have membrane-protective and antidenaturing effects (Liang *et al.*, 2013; Kolupaev *et al*., 2019; Deryabin, Trunova, 2021). The pattern of accumulation of stress metabolites in plants is known to change under the influence of melatonin (Shi *et al*., 2015; Jiang *et al.*, 2016; Tiwari *et al.,* 2021; Zafar *et al*., 2020; Sattar *et al*., 2023). However, the reported effects can vary. For example, both increases (Antoniou *et al.*, 2017; Ahmad *et al*., 2021; Awan *et al*., 2023) and decreases (Zamani *et al.*, 2019; Buttar *et al.,* 2020) in proline content under the influence of melatonin have been reported in plants of different species under drought. In addition to the multiple effects of increased sugars in plants when treated with melatonin under stress conditions, soybean plants were found to have decreased soluble carbohydrates under drought conditions (Imran *et al*., 2021).

The effects of melatonin may depend not only on the species but also on the varietal characteristics of the plants. Thus, melatonin was shown to enhance proline accumulation under drought in non-resistant wheat cultivars and to attenuate it in resistant cultivars (Li *et al*., 2020). It is known that the adaptive strategies of wheat varieties and other cereal crops can differ significantly, which can be manifested in the different contribution of enzymatic and non-enzymatic components of the antioxidant system to adaptation to oxidative stress (Kolupaev *et al*., 2023a; 2023c).

Despite the importance of wheat as a food crop, there has been very little research on the effect of melatonin on wheat drought tolerance (Cui *et al*., 2018; Li *et al*., 2020; Zhang *et al*., 2023). The information on the peculiarities of the manifestation of stress-protective effects of melatonin in varieties with different drought adaptation strategy is particularly fragmentary. In particular, there are no works that have simultaneously studied the effect of melatonin on the functioning under stress conditions of antioxidant and osmoprotective systems in etiolated seedlings of wheat varieties differing in drought tolerance.

Meanwhile, the study of melatonin regulation of adaptive processes in winter wheat at the earliest stages of development has not only theoretical but also applied significance, as severe droughts in autumn are a typical phenomenon in Eastern European countries, including Ukraine (Romanenko *et al.,* 2018). In this regard, the aim of the work was to compare the reactions of stress-protective systems of 2-4-day-old wheat seedlings of Tobak (drought-tolerant) and Doskonala (sensitive) varieties to melatonin action under model drought conditions (treatment with 15% PEG 6000).

MATERIAL AND METHODS

Plant materials and treatments

Wheat seedlings of the varieties Tobak (characterized by high drought tolerance under laboratory (Kolupaev *et al.,* 2023c) and field conditions (Urban *et al.,* 2018), originator—Saaten-Union GmbH, Isernhagen HB, Germany) and Doskonala (sensitive to drought (Kolupaev *et al*., 2023c), originator—Yuriev Plant Production Institute of NAAS of Ukraine) were used for research. Seeds of reproduction of 2022, obtained in the National Center for Plant Genetic Resources of Ukraine (Kharkiv, eastern forest-steppe of Ukraine), were used in this work.

Seeds were disinfected in 70% ethanol for 2 min, then transferred to 2% sodium hypochlorite solution for 15 min, washed 10 times with sterile distilled water, and germinated on water in Petri dishes in a thermostat at 24°C without light for 2 days. After that, seedlings of approximately the same length were transferred to Petri dishes with two layers of filter paper moistened with 15% PEG 6000 solution. Control seedlings were transferred to Petri dishes with filter paper moistened with distilled water. Melatonin was dissolved in a small volume of ethanol, the solutions were diluted with distilled water and added to Petri dishes to obtain working solutions with concentrations in the range of 0.2-5.0 μM. In special experiments it was found that ethanol in concentrations not higher than 0.001% applied with melatonin solutions did not affect the growth of wheat seedlings and their resistance to osmotic stress.

After two days of exposure of seedlings to PEG 6000 and/or melatonin solutions, shoot and root biomass of seedlings were evaluated and biochemical parameters in shoots were determined.

Measurement of hydrogen peroxide content

To determine 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). The concentration of H_2O_2 in the supernatant was determined by the ferrothiocyanate method (Sagisaka, 1976) with slight modifications. For this purpose, 0.5 ml of 2.5 M NH_4SCN, 0.5 ml of 50% TCA, 1.5 ml of supernatant, and 0.5 ml of 10 mM ammonium ferrous sulfate were added to tubes. After mixing, the samples were transferred to cuvettes and the absorbance at 480 nm was determined.

Evaluation of LPO products content

To analyze the amount of lipid peroxidation products (LPO) reacting with 2-thiobarbituric acid (mainly malonic dialdehyd-MDA), shoots were homogenized in a reaction medium containing 0.25% 2-thiobarbituric acid in 10% TCA, the homogenate in tubes covered with foil lids was placed in a boiling bath for 30 min. The samples were then cooled and centrifuged at 10000 g for 15 min. The absorbance of the supernatant was determined at 532 nm (maximum light absorption of MDA) and 600 nm (to correct for non-specific light absorption) (Kolupaev *et al.,* 2021).

Analysis of antioxidant enzyme activity

To determine the activity of the antioxidant enzymes SOD, catalase, and guaiacol peroxidase, shoots were homogenized on cold in 0.15 M K, Na-phosphate buffer (pH 7.6) containing EDTA (0.1 mM) and dithiothreitol (1 mM) (Kolupaev *et al.,* 2022). Enzyme activity was determined in the supernatant after centrifugation of the homogenate at 8000 g for 10 min at 4°C. Since the water content in shoots of seedlings of different variants differed during sampling, the studied parameters were calculated per gram of dry weight.

SOD activity (EC 1.15.1.1.1) was determined at pH 7.6 by a method based on the ability of the enzyme to compete with nitroblue tetrazolium for superoxide anions formed by aerobic interaction of NADH and phenazine methosulfate; absorbance was determined at 540 nm (Kolupaev *et al*., 2012). The activity of catalase (EC 1.11.1.6) was analyzed at pH 7.0 by the amount of hydrogen peroxide decomposed per unit time. The activity of guaiacol peroxidase (EC 1.11.1.7) was determined using guaiacol as hydrogen donor and hydrogen peroxide as substrate. In this case, the pH of the reaction mixture was preliminarily adjusted to 6.2 with K, Na-phosphate buffer (Kolupaev *et al*., 2022). The absorbance of tetraguaiacol was determined at 470 nm.

Estimation of low-molecular-weight protectors

The total sugar content in the plant material was determined by the Morris-Roe method on the basis of the anthrone reagent (Zhao *et al*., 2003) in our modification described earlier (Kolupaev *et al*., 2023c). D-glucose was used as a standard.

Proline content was determined using the ninhydrin reagent (Bates *et al*., 1973). L-proline was used as the standard.

For the determination of total phenolics and flavonoids, seedlings were homogenized in 80% ethanol, extracted for 20 min at room temperature, and centrifuged at 8000×g for 15 min. For the analysis of phenolic compounds, 0.5 ml of supernatant, 8 ml of distilled water and 0.5 ml of Folin reagent were added to reaction tubes, stirred and after 3 min, 1 ml of 10% sodium carbonate was added. The absorbance of the reaction mixture was measured at 725 nm after 1 h (Bobo-García *et al*., 2015). The phenolic content was expressed as μmol gallic acid per gram dry weight.

Before determining the content of anthocyanins and UV-B absorbing flavonoids, the supernatant was acidified with HCl to a final concentration of 1%. The absorbance of the solutions was determined at 530 and 300 nm (Nogués, Baker, 2000). Results were expressed as absorbance per dry weight of plant material.

Experimental replication and statistical analysis

Experiments had 3-4 biological replicates. For seedling organ weight determination, each sample consisted of 30 seedlings. Samples for biochemical analyses consisted of 12 seedlings each. Data for each parameter were statistically analyzed using Fisher's analysis (ANOVA and Least Significant Difference (LSD) test at 5% probability level). Figures and tables show means and their standard errors; different letters indicate values whose differences are significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth response of wheat seedlings to drought action and melatonin treatment

Under the influence of drought created by PEG 6000, the growth of root and shoot biomass decreased by about 35% in the resistant variety Tobak (Figure 1). At the same time, in the drought-sensitive variety Doskonala, under the conditions of model drought, the indicators of seedling organ biomass decreased by about 53%.

Treatment with melatonin at concentrations of 0.2-5.0 μM significantly attenuated the growth inhibitory effect of osmotic stress (Figure 1). The most effective concentration was 1 μM. At the same time, varietal differences were observed: the positive effect of melatonin on root and shoot growth under osmotic stress was more pronounced in the Doskonala variety than in the Tobak one (Figure 1).

Melatonin modulation of oxidative stress effects under drought conditions

Incubation of seedlings on medium containing PEG 6000 increased the hydrogen peroxide content by about 35% in the resistant cultivar Tobak and by 1.5-fold in the drought-sensitive cultivar Doskonala (Figure 2, A). Melatonin treatment in both varieties under stress conditions reduced the hydrogen peroxide content in the shoots almost to the control level.

Under drought conditions, the content of TBA-active LPO products increased by almost 30% in Tobak and by 70% in Doskonala (Figure 2, B). Under the influence of melatonin treatment, this effect decreased to 35% in the non-tolerant Doskonala variety. At the same time, the decrease in the content of LPO products in the presence of melatonin was less pronounced in the Tobak variety.

Activity of antioxidant enzymes

Basal SOD activity in the resistant variety Tobak was 2 times higher than in the non-resistant variety Doskonala (Table 1). Under drought stress, SOD activity decreased in both varieties. Melatonin treatment alleviated this effect in

Tobak. In Doskonala, however, the enzyme activity increased significantly under the influence of melatonin, exceeding the values not only of the variety treated with PEG 6000, but also of the control.

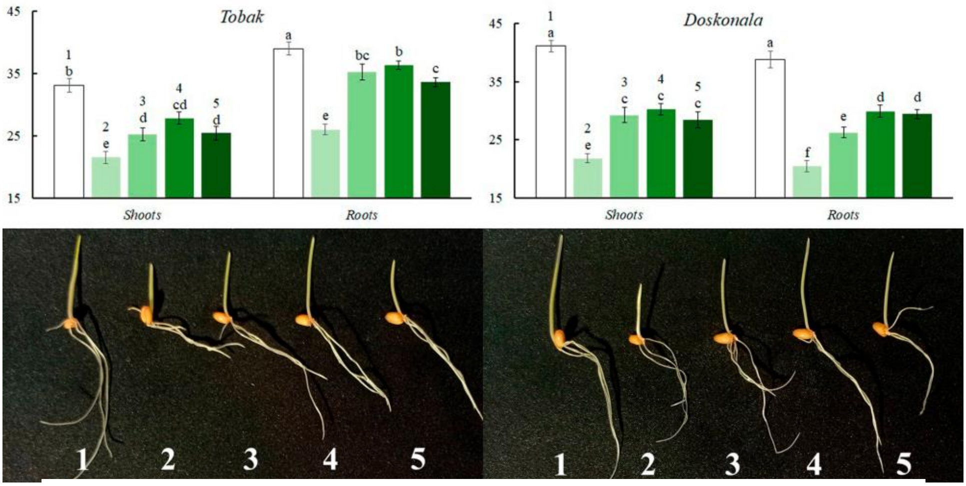

Figure 1. Weight of roots and shoots (mg) of wheat seedlings of Tobak and Doskonala varieties under the effect of PEG 6000 and melatonin. 1—control; 2—PEG (15%); 3—PEG (15%)+melatonin (0.2 μM); 4—PEG (15%)+melatonin (1 μM); 5—PEG (15%)+melatonin (5 μM).

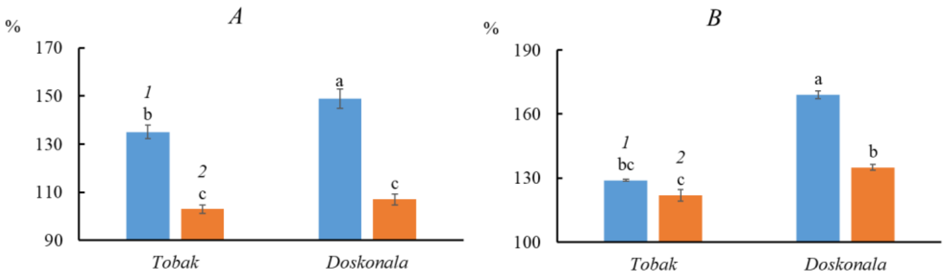

Figure 2. Content (% of control) of hydrogen peroxide (A) and MDA (B) in shoots of wheat seedlings. 1—PEG 6000 (15%); 2—PEG 6000 (15%)+melatonin (1 μM).

The catalase activity of the resistant variety Tobak did not change significantly under model drought, nor did melatonin treatment significantly affect this index (Table 1). At the same time, the non-resistant variety Doskonala showed a significant decrease in catalase activity under stress; concurrent treatment with melatonin slightly alleviated this effect.

The activity of guaiacol peroxidase in response to osmotic stress decreased both in Doskonala and (to a lesser extent) in Tobak varieties. Under the influence of melatonin, a tendency towards stabilization of the enzyme activity was observed in both varieties tested (Table 1).

Table 1. Activity of SOD, catalase, and guaiacol peroxidase in the shoots of wheat seedlings

Experimental variant	SOD activity (U/g DW min)	Catalase activity (mmol H_2O_2/g DW min)	Guaiacol peroxidase activity (U/g DW min)
Tobak			
Control	62.6±1.6 a	10.8±0.2 c	7.64±0.05 c
PEG 6000 (15%)	35.6±1.8 c	10.3±0.2 c	6.81±0.06 e
PEG 6000 (15%) + Melatonin (1 μM)	48.6±1.1 b	11.0±0.2 c	7.43±0.08 cd
Doskonala			
Control	30.1±1.7 d	15.8±0.5 a	9.76±0.08 a
PEG 6000 (15%)	20.4±0.7 e	11.9±0.3 bc	7.26±0.03 d
PEG 6000 (15%) + Melatonin (1 μM)	44.4±1.0 b	12.7±0.2 b	8.62±0.06 b

Content of low-molecular-weight protective compounds

The basal proline content in shoots of seedlings of the resistant variety Tobak was significantly lower than that of the susceptible variety Doskonala (Figure 3, A). It increased proportionally in both varieties in response to the action of model drought. Melatonin treatment of the Tobak had almost no effect on the character of changes in proline content under stress. At the same time, melatonin treatment of Doskonala under the action of melatonin significantly increased proline content in shoots of seedlings under osmotic stress.

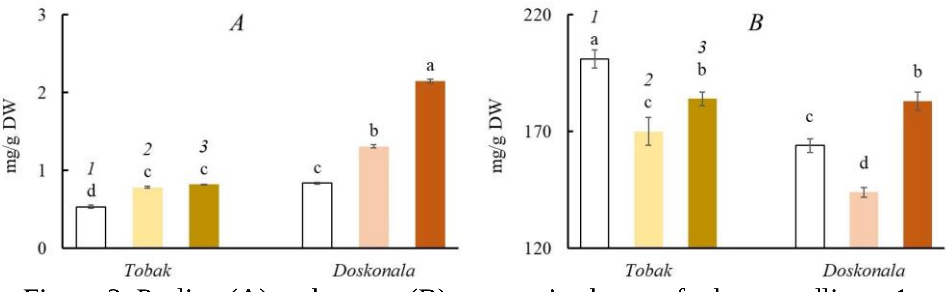

Figure 3. Proline (A) and sugars (B) content in shoots of wheat seedlings. 1—control; 2—PEG 6000 (15%); 3—PEG 6000 (15%)+melatonin (1 μM).

The constitutive sugar content of Tobak was significantly higher than that of Doskonala (Figure 3, B). Under the influence of model drought, both varieties showed a proportional decrease in their amounts.

The melatonin treatment partially offset this effect of drought in Tobak, while its effect was even more pronounced in Doskonala: in the variant with a combination of melatonin and stress, the sugars content exceeded the control values.

The basic total content of phenolic compounds in the shoots of seedlings of the two varieties did not differ significantly (Table 2). Under stress, this index slightly increased in the resistant variety Tobak and did not change in the drought-sensitive variety Doskonala. Melatonin treatment caused a tendency to increase the content of phenolic compounds in Doskonala, but this effect was not significant at $P \leq 0.05$.

Table 2. Content of secondary metabolites in wheat seedlings

Experimental variant	Total content of phenolic compounds (μmoles of gallic acid/g DW)	Anthocyanins content (A_{530}/g DW)	Flavonoids content (A_{300}/g DW)
Tobak			
Control	9.48±0.23 b	0.255±0.003 a	2.05±0.03 b
PEG 6000 (15%)	10.20±0.11 a	0.185±0.003 c	1.55±0.03 d
PEG 6000 (15%) + Melatonin (1 μM)	9.88±0.23 ab	0.220±0.005 b	1.71±0.02 cd
Doskonala			
Control	9.87±0.13 ab	0.250±0.011 a	2.21±0.05 a
PEG 6000 (15%)	9.67±0.25 ab	0.140±0.005 d	1.65±0.05 d
PEG 6000 (15%) + Melatonin (1 μM)	10.30±0.12 a	0.210±0.011 b	1.84±0.02 c

The anthocyanin content under osmotic stress decreased in both varieties, but more significantly in the non-resistant Doskonala. Melatonin treatment largely prevented this stress effect in both (Table 2). Similar changes occurred in the flavonoid content in both varieties: it decreased under osmotic stress, while melatonin treatment mitigated this effect.

DISCUSSION

Under model drought conditions, melatonin treatment had a significant stress-protective effect on seedlings of both wheat varieties studied. These effects were expressed in the alleviation of stress-induced inhibition of root and shoot growth (Figure 1) and in the reduction of the manifestation of oxidative cell damage (Figure 2).

The results obtained and the information available in the literature allow to discuss several mechanisms of melatonin influence on the resistance of seedlings to osmotic stress. It is known that one of the early negative effects of drought is the excessive formation of ROS associated with the disruption of electron transport processes in chloroplasts and mitochondria. Insufficient water supply increases ROS production not only due to excessive reduction of components of the electron transport chain in chloroplasts, which occurs as a result of stomatal closure due to limited carbon dioxide supply (de Carvalho, 2008), but also due to the significant contribution of mitochondria to oxidative damage processes plants under drought conditions. For example, under stress conditions in wheat leaves, the content of carbonylated proteins in mitochondria was an order of magnitude higher than in chloroplasts (Bartoli *et al.*, 2004). It can be assumed that the mechanisms of oxidative damage unrelated to the functioning of the photosynthetic apparatus were dominant in the model objects we used, etiolated seedlings. It should be noted that under drought conditions it is possible to accumulate ROS due to a decrease in the activity of antioxidant enzymes (de Carvalho, 2008). Under the conditions of our experiments, such effects were manifested with respect to the main antioxidant enzymes, whose activity decreased particularly markedly in the non-tolerant cultivar Doskonala (Table 1).

Due to the significant contribution of oxidative stress to the development of drought damage, melatonin, which is a direct and indirect antioxidant (Kobylińska *et al.*, 2018), is considered as a new effective tool for the management of drought tolerance in plants (Ayyaz *et al*., 2022; Gu *et al.*, 2022).

The decrease in the amount of ROS in cells under the influence of melatonin may be related to its direct antioxidant effect (Ayyaz *et al*., 2022). However, the manifestation of the stress-protective effect of melatonin at low (in our case, micromolar) concentrations does not give reason to consider this mechanism as the main one. The mechanisms related to the involvement of melatonin in the complex signal-regulatory network of cells, including signaling processes involving ROS, may be much more likely (Gu *et al*., 2022). Thus, there is evidence for increased expression of genes of the catalytic subunit of NADPH oxidase under the influence of melatonin in tomato (Mukherjee, 2019). The associated increase in H_2O_2 levels may positively regulate the activity of antioxidant enzymes (SOD, catalase, peroxidase) as well as the expression of other stress-induced genes (in particular, mitogen-activated protein kinase 1, thermosperminsintase, and heat shock proteins) (Mukherjee, 2019). In general, melatonin treatment has been found to increase the activity of many antioxidant enzymes (including SOD, peroxidase, ascorbate peroxidase, dehydroascorbate reductase, glutathione S-transferase, glutathione reductase, and others) in plants of different taxonomic groups, including maize, tomato, citrus, and soybean (Gu *et al.*, 2022). Priming of wheat seeds with melatonin caused a significant increase in catalase activity under subsequent heat stress (Kolupaev *et al*., 2023d). Under the same conditions, melatonin reversed the heat stress-induced decrease in guaiacol peroxidase activity. The present work shows stabilization of SOD and guaiacol peroxidase activity in wheat seedlings under osmotic stress by melatonin treatment (Table 1). It should be noted that a more significant modulation of antioxidant enzyme activity by melatonin was observed in the drought-sensitive cultivar Doskonala.

Other ways to stabilize the homeostasis of wheat seedling cells under the action of melatonin may be related to the activation of the synthesis of various low-molecular compounds that exhibit multifunctional stress-protective effects. Under the conditions of our experiments, melatonin treatment significantly enhanced proline accumulation in Doskonala wheat seedlings under drought conditions (Figure 3, A). At the same time, melatonin had little effect on proline content under stress conditions in Tobak variety. These results are consistent with data from Chinese cultivars (Li *et al.,* 2020). This work showed that melatonin enhanced proline accumulation in a non-drought-tolerant cultivar and attenuated this effect in a drought-tolerant cultivar. A decrease in stress-induced proline accumulation under melatonin treatment was also found in Trig*onella foenum-gracum* plants (Zamani *et al.,* 2019). In general, most of the studies conducted on plants of different species show that melatonin increases proline content under stresses associated with cell dehydration (Buttar *et al.,* 2020; Tiwari *et al*., 2021; Awan *et al.,* 2023). An increase in activity and enhanced gene expression of proline synthesis enzymes under the influence of exogenous melatonin has also been reported (Antoniou *et al*., 2017). However, it should be noted that proline accumulation can be considered as a sign of a rather severe stress effect. For example, a high correlation between the accumulation of proline and the LPO product MDA under heat stress (r=0.91) was found in etiolated seedlings of seven wheat varieties (Kolupaev *et al.,* 2023c). Apparently, the stress used in this work was critical for the non-tolerant variety Doskonala. At the same time, the resistant variety Tobak maintained a relatively stable proline content (Figure 3, A). It is probable that melatonin activates different stress-protective reactions in wheat seedlings, and the extent of its protective effect relies on the adaptive strategies of species and varieties.

Drought caused a decrease in sugar content in seedlings of both varieties, apparently due to their increased consumption for respiration and other metabolic processes under stress conditions (Figure 3, B). Melatonin treatment promoted the stabilization of their content under osmotic stress, especially in the sensitive variety Doskonala. Further investigation is required to comprehend the mechanisms of this impact. However, literature suggests that melatonin treatment can affect carbohydrate metabolism in plant cells. For instance, in Bermuda grass plants under drought conditions, sugar content equalization was observed during melatonin treatment (Shi *et al*., 2015). In buckwheat, melatonin-treated plants showed a 60% increase in sugar content compared to untreated plants (Tiwari *et al.,* 2021). It has also been shown that exogenous melatonin under stress conditions can modulate the concentration of sugars in etiolated cells of the mutant (chlorophyll-free) form of tobacco, Bright Yellow 2 (Kobylińska *et al.*, 2018). The authors believe that melatonin shifts cell metabolism along the gluconeogenesis pathway, allowing the synthesis of carbohydrates from precursors that are not sugars. However, in etiolated cereal seedlings, another mechanism for the enhancement of carbohydrate metabolism by melatonin is more likely to be related to the activation of polysaccharide hydrolysis in the grain and the increased entry of sugars into the shoot (Lei *et al.,* 2021).

Both proline and sugars are not only osmolytes, but also have antidenaturation and membrane-protective properties (Liang *et al*., 2013; Deryabin, Trunova, 2021). In addition, proline and sugars are quite potent antioxidants (Kolupaev *et al.,* 2019).

Melatonin also affects secondary metabolism in plants. Under the conditions of our experiments, treatment of seedlings with melatonin did not significantly affect the total content of phenolic compounds (Table 2). At the same time, such treatment under osmotic stress conditions promoted the preservation of the pool of polyphenols, anthocyanins and flavonoids absorbing in the UV-B region. It should be noted that rapid degradation of polyphenolic compounds under stress conditions was also observed in other works (Zafar *et al*., 2020). At the same time, melatonin has the ability to enhance the accumulation of anthocyanins and other polyphenols with strong antioxidant activity. For example, a ROS-dependent enhancement of anthocyanin synthesis in pear fruit by melatonin has been demonstrated (Sun *et al.,* 2021). It is noteworthy that in Bermuda grass, the function of two genes controlling the expression of phenylalanine ammonia-lyase (a key enzyme for the synthesis of phenolic compounds) is suppressed during drought stress, but increased after treatment with exogenous melatonin (Ayyaz *et al*., 2022). Foliar treatment with melatonin on *Fagopyrum tataricum* plants resulted in a significant increase in the activity of phenylalanine ammonia-lyase and flavonoid content under drought conditions (Hossain *et al.,* 2020). Melatonin treatment also led to the synthesis of polyphenolic compounds in various plant species under UV-B stress conditions (Plokhovska *et al*., 2023).

CONCLUSIONS

Melatonin treatment had a positive effect on biomass accumulation by wheat seedlings under osmotic stress. At the same time, it significantly attenuated oxidative damage by affecting various components of the antioxidant system. Under the effect of melatonin, the activities of key enzymes SOD and guaiacol peroxidase, as well as the amount of sugars, anthocyanins, and flavonoids absorbing in UV-B, stabilized. Melatonin treatment during model drought conditions resulted in a significant increase in proline content in non-tolerant wheat cultivar. In general, melatonin had a more pronounced stress-protective effect on seedlings of drought-sensitive wheat variety. Thus, the stress-protective effect of melatonin is associated with normalization of antioxidant and osmoprotective systems functioning under drought, and it depends to some extent on varietal characteristics of adaptive strategies.

Treatment with melatonin can be considered as a promising technique for increasing the drought resistance of wheat in the early stages of development. However, for the actual use of this technique in plant growing, special research is required, in particular, an assessment of the sensitivity of varieties to the stress-protective effect of melatonin. Of course, a separate cycle of research is necessary to develop a rational technological method for treating plants or seeds priming with melatonin.

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REFERENCES

Ahmad, S., Muhammad, I., Wang, G. Y., Zeeshan, M., Yang, L., Ali, I., & Zhou, X. B. (2021). Ameliorative effect of melatonin improves drought tolerance by regulating growth, photosynthetic traits and leaf ultrastructure of maize seedlings. BMC Plant Biology, 21(1), 368. https://doi.org/10.1186/s12870-021-03160-w

Akula, R., & Mukherjee, S. (2020). New insights on neurotransmitters signaling mechanisms in plants. Plant Signaling & Behavior, 15(6):1737450, https://doi.org/10.1080/15592324.2020.1737450

Antoniou, C., Chatzimichail, G., Xenofontos, R., Pavlou, J. J., Panagiotou, E., Christou, A., & Fotopoulos, V. (2017). Melatonin systemically ameliorates drought stress-induced damage in Medicago sativa plants by modulating nitro-oxidative homeostasis and proline metabolism. Journal of Pineal Research, 62:e12401. https://doi.org/10.1111/jpi.12401

Arnao, M. B., & Hernández-Ruiz, J. (2013). Growth conditions determine different melatonin levels in *Lupinus albus* L. Journal of Pineal Research, 55:149-155.

Arnao, M. B., & Hernandez-Ruiz, J. (2015). Functions of melatonin in plants: a review. Journal of Pineal Research, 59:133-150.

Awan, S. A., Khan, I., Wang, Q., Gao, J., Tan, X. & Yang, F. (2023). Pre-treatment of melatonin enhances the seed germination responses and physiological mechanisms of soybean (*Glycine max* L.) under abiotic stresses. Frontiers in Plant Science, 14:1149873. https://doi.org/10.3389/fpls.2023.1149873

Ayyaz, A., Shahzadi, A. K., Fatima, S., Yasin, G., Zafar, Z. U., Athar, H. R., & Farooq, M. A. (2022). Uncovering the role of melatonin in plant stress tolerance. Theoretical and Experimental Plant Physiology, 34:335-346.

Bartoli, C. G., Gomez, F., Martinez, D. E. & Guiamet, J. J. (2004). Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). Journal of Experimental Botany, 55:1663-1669.

Bates, L. S., Walden, R. P. & Tear, G. D. (1973). Rapid determination of free proline for water stress studies. Plant & Soil, 39:205-210.

Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intralaboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. Journal of the Science of Food and Agriculture, 95:204-209.

Buttar, Z. A., Wu, S. N., Arnao, M. B., Wang, C., Ullah, I., & Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. Plants. 9(7):809. https://doi.org/10.3390/plants9070809

Cui, G., Sun, F., Gao, X., Xie, K., Zhang, C., Liu, S., & Xi, Y. (2018). Proteomic analysis of melatonin-mediated osmotic tolerance by improving energy metabolism and autophagy in wheat (*Triticum aestivum* L.). Planta, 248 (1), 69-87.

Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and Wwheat production. PLoS One. 11(5):e0156362. https://doi.org/10.1371/journal.pone.0156362.

de Carvalho, M. H. C. (2008). Drought stress and reactive oxygen species. Production, scavenging and signaling. Plant Signaling and Behavior, 3:156-165.

Deryabin, A. N., & Trunova, T. I. (2021). Colligative effects of solutions of low-molecular sugars and their role in plants under hypothermia. Biology Bulletin, 48(3):S29-S37. https://doi.org/10.1134/S1062359021060042

Dubbels, R., Reiter, R.J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., Schiwara, H.W., & Schloot, W. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. Journal of Pineal Research, 18:28-31.

Fan, J., Xie, Y., Zhang, Z., & Chen, L. (2018). Melatonin: A multifunctional factor in plants. International Journal of Molecular Sciences, 19:1528. https://doi.org/10.3390/ijms19051528

Gu, Q., Xiao, Q., Chen, Z., & Han, Y. (2022). Crosstalk between melatonin and reactive oxygen species in plant abiotic stress responses: An updateInternational Journal of Molecular Sciences, 23:5666. https://doi.org/10.3390/ijms23105666

Hossain, M. S., Li, J., Sikdar, A., Hasanuzzaman, M., Uzizerimana, F., Muhammad, I., Yuan, Y., Zhang, C., Wang, C., & Feng, B. (2020). Exogenous melatonin modulates the physiological and biochemical mechanisms of drought tolerance in Tartary Buckwheat (*Fagopyrum tataricum* (L.) Gaertn). Molecules, 25(12):2828. https://doi.org/10.3390/molecules25122828

Imran, M., Latif Khan, A., Shahzad, R., Aaqil Khan, M., Bilal, S., Khan, A., Kang, S. M., & Lee, I. J. (2021). Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. AoB PLANTS, 13(4):plab026. https://doi.org/10.1093/aobpla/plab026

Jogawat, A., Yadav, B., Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. Physiologia Plantarum, 172(2):1106-1132.

Joseph, E. A., Radhakrishnan, V. V., & Mohanan, K. V. (2015). A study on the accumulation of proline-an osmoprotectant amino acid under salt stress in some native rice cultivars of North Kerala, India. Universal Journal of Agricultural Research, 3(1):15-22. https://doi.org/10.13189/ujar.2015.030104

Kobylińska, A., Borek, S., & Posmyk, M. M. (2018). Melatonin redirects carbohydrates metabolism during sugar starvation in plant cells. Journal of Pineal Research, 64(4):e12466. https://doi.org/10.1111/jpi.12466

Kolupaev, Y. E., Karpets, Y. V. & Kabashnikova, L. F. (2019). Antioxidative system of plants: Cellular compartmentalization, protective and signaling functions, mechanisms of regulation (Review). Applied Biochemistry and Microbiology, 55(5):441-459.

Kolupaev, Yu. E., Kokorev, A. I., Shkliarevskyi, M. A. Lugovaya A. A., Karpets Yu. V., & Ivanchenko O. E. (2021). Role of NO synthesis modification in the protective effect of putrescine in wheat seedlings subjected to heat stress. Applied Biochemistry and Microbiology, 57(3):384-391.

Kolupaev, Yu. E., Taraban, D. A., Karpets, Yu. V., Makaova, B. E., Ryabchun, N. I., Dyachenko, A. I. & Dmitriev, O. P. (2023d). Induction of cell protective reactions of *Triticum aestivum* and *Secale cereale* to the effect of high temperatures by melatonin. Cytology and Genetics, 57(2):117-127.

Kolupaev, Yu. E., Yastreb, T. O., & Dmitriev, A. P. (2023b). Signal mediators in the implementation of jasmonic acid's protective effect on plants under abiotic stresses. Plants. 12(14):2631. https://doi.org/10.3390/plants12142631

Kolupaev, Yu. E., Yastreb, T. O., Shvidenko, N. V., & Karpets, Yu. V. (2012). Induction of heat resistance of wheat coleoptiles by salicylic and succinic acids: Connection of the effect with the generation and neutralization of reactive oxygen species. Applied Biochemistry and Microbiology, 48(5):500-505.

Kolupaev, Yu. E., Makaova, B. E., Ryabchun, N. I., Kokorev, A. I., Sakhno, T. V., Sakhno, Y., Yastreb, T. O., & Marenych, M. M. (2022). Adaptation of cereal seedlings to oxidative stress induced by hyperthermia. Agriculture and Forestry, 68(4):7-18. https://doi.org/10.17707/AgricultForest.68.4.01

Kolupaev, Yu. E.. Yastreb, T. O., Ryabchun, N. I., Kuzmyshyna, N.V., Shkliarevskyi, M. A., Barabolia, O., & Pysarenko, V. M. (2023c). Response of Triticum aestivum seedlings of different ecological and geographical origin to heat and drought: relationship with resistance to oxidative stress and osmolyte accumulation. Agriculture and Forestry, 69(2):83-99. https://doi.org/10.17707/AgricultForest.69.2.07

Kolupaev. Yu. E, Yastreb, T. O., Ryabchun, N. I., Kokorev, A. I., Kolomatska, V. P., & Dmitriev, A. P. (2023a). Redox homeostasis of cereals during acclimation to drought. Theoretical and Experimental Plant Physiology, 35(2):133-168.

Kosakivska, I. V., Vedenicheva, N. P., Babenko, L. M., Voytenko, L. V., Romanenko, K. O., & Vasyuk, V. A. (2022). Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. Molecular Biology Reports, 49(1):617-628.

Lei, K., Sun, S., Zhong, K., Li, S., Hu, H., Sun, C., Zheng, Q., Tian, Z., Dai, T., Sun, J. (2021) Seed soaking with melatonin promotes seed germination under chromium stress via enhancing reserve mobilization and antioxidant metabolism in wheat. Ecotoxicology and Environmental Safety, 220:112241. https://doi.org/10.1016/j.ecoenv.2021.112241.

Li, D., Batchelor, W.D., Zhang, D., Miao, H., Li, H., Song, S., & Li, R. (2020). Analysis of melatonin regulation of germination and antioxidant metabolism in different wheat cultivars under polyethylene glycol stress. PLOS ONE, 15(8): e0237536. https://doi.org/10.1371/journal.pone.0237536

Liang, X., Zhang, L., Natarajan, S. K., & Becker, D. F. (2013) Proline mechanisms of stress survival. Antioxidants & Redox Signaling, 19(9):998-1011.

Ma, D., Sun, D., Wang, C., Li, Y., & Guo, T. (2014). Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress. Plant Physiology and Biochemistry, 80:60-66.

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T.C., Garcia-Sanchez, F., Rubio, F., Nortes, P.A., Mittler, R., & Rivero, R.M. (2018). Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. Molecules, 23(3):535. https://doi.org/10.3390/molecules23030535

Mukarram, M., Choudhary, S., Kurjak, D., Petek, A., & Khan, M. M. A. (2021). Drought: sensing, signalling, effects and tolerance in higher plants. Physiologia Plantarum, 172(2):1291-1300.

Mukherjee, S. (2019). Insights into nitric oxide-melatonin crosstalk and N-nitrosomelatonin functioning in plants. Journal of Experimental Botany, 70: 6035-6047.

Nogués, S., & Baker, N. R. (2000). Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. Journal of Experimental Botany, 51:1309-1317.

Pržulj, N., Jovović, Z. & Velimirović, A. (2020). Breeding small grain cereals for drought tolerance in a changing climate. Agriculture and Forestry, 66(2):109-123. https://doi.org/10.17707/AgricultForest.66.2.11

Ribeiro, D. G. S. da Silva, B. R. S., & Lobato, A. K. S. (2019). Brassinosteroids induce tolerance to water deficit in soybean seedlings: contributions linked to root anatomy and antioxidant enzymes. Acta Physiologiae Plantarum, 41:82. https://doi.org/10.1007/s11738-019-2873-2

Romanenko, O., Kushch, I., Zayets, S., & Solodushko, M. (2018). Viability of seeds and sprouts of winter crop varieties under drought conditions of Steppe. Agroecological Journal, 1:87-95.

Rouhnezhad, S., Janmohammadi, M., Sabaghnia, N., & Nasiri, Y. (2023). The effects of seed invigoration treatments on wheat seedling growth and activity of enzymatic antioxidants. Agriculture and Forestry, 69(2):21-34. https://doi.org/10.17707/AgricultForest.69.2.02

Sagisaka, S. (1976). The occurrence of peroxide in a perennial plant, *Populus gelrica*. Plant Physiology, 57:308-309.

Sattar, A., Sher, A., Ijaz, M., Ul-Allah, S., Hussain, S., Rasheed, U., Hussain, J., Al-Qahtani, S. M., Al-Harbi, N. A., Mahmoud, S. F., & Ibrahim M. F. M. (2023). Modulation of antioxidant defense mechanisms and morphophysiological attributes of wheat through exogenous application of silicon and melatonin under water deficit conditions. Sustainability, 15:7426. https://doi.org/10.3390/su15097426

Shi, H., Jiang, C., Ye, T., Tan, D. X., Reiter, R. J., Zhang, H., Liu, R., & Chan, Z. (2015). Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. Journal of Experimental Botany, 66(3): 681-694.

Singh, S., Prakash, P., & Singh, A. K. (2021). Salicylic acid and hydrogen peroxide improve antioxidant response and compatible osmolytes in wheat (*Triticum aestivum* L.) under water deficit. Agricultural Research, 10(2):175-186.

Sun, H., Cao, X., Wang, X., Zhang, W., Li, W., Wang, X., Liu, S., & Lyu, D. (2021). RBOH-dependent hydrogen peroxide signaling mediates melatonin-induced anthocyanin biosynthesis in red pear fruit. Journal of Plant Sciences, 313:111093. https://doi.org/10.1016/j.plantsci.2021.111093

Sun, L., Li, X., Wang, Z., Sun, Z., Zhu, X., Liu, S., Song, F., Liu, F., & Wang, Y. (2018). Cold priming induced tolerance to subsequent low temperature stress is enhanced by melatonin application during recovery in wheat. Molecules, 23(5):1091. https://doi.org/10.3390/molecules23051091

Tiwari, R. K., Lal, M. K., Kumar, R., Chourasia, K. N., Naga, K. C., Kumar, D., Das, S. K., & Zinta, G. (2021). Mechanistic insights on melatonin-mediated drought stress mitigation in plants. Physiologia Plantarum, 172(2):1212-1226.

Urban, O., Hlaváčová, M., Klem, K., Novotná, K., Rapantová, B., Smutná, P., Horáková, V., Hlavinka, P., Škarpa, P. & Trnka, M. (2018). Combined effects of drought and high temperature on photosynthetic characteristics in four winter wheat genotypes. Field Crops Research, 223:137-149.

Vignesh, M. R., & Palanisamy, S. (2021). Aquaporin and its effect on foliar uptake to overcome drought stress in plants. Plant Physiology Reports, 26(2):193-199.

Ye J, Wang S, Deng X, Yin L, Xiong B, & Wang X (2016). Melatonin increased maize (*Zea mays* L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. Acta Physiologiae Plantarum, 38(2):1-13.

Yemets, A. I., Karpets, Yu. V., Kolupaev, Yu. E., & Blume, Y. B. (2019). Emerging technologies for enhancing ROS/RNS homeostasis. In: Reactive oxygen, nitrogen and sulfur species in plants: Production, metabolism, signaling and defense mechanisms, vol. 2. (John Wiley & Sons Ltd), 873-922. https://doi.org/10.1002/9781119468677.ch39

Zafar, S., Akhtar, M., Perveen, S., Hasnain, Z., & Khalil, A. (2020). Attenuating the adverse aspects of water stress on wheat genotypes by foliar spray of melatonin and indole-3-acetic acid. Physiology and Molecular Biology of Plants: An International Journal of Functional Plant Biology, 26(9):1751-1762.

Zamani, Z., Amiri, H., & Ismaili, A. (2019). Improving drought stress tolerance in fenugreek (*Trigonella foenum-graecum*) by exogenous melatonin. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology, 154(5):643-655.

Zhang, Z., Guo, L., Sun, H., Wu, J., Liu, L., Wang, J., Wang, B., Wang, Q., Sun, Z., & Li, D. (2023). Melatonin increases drought resistance through regulating the fine root and root hair morphology of wheat revealed with RhizoPot. Agronomy, 13, 1881. https://doi.org/10.3390/agronomy13071881

Zhao, G., Zhao, Y., Yu, X., Kiprotich, F., Han, H., Guan, R., Wang, R., & Shen, W. (2018). Nitric oxide is required for melatonin-enhanced tolerance against salinity stress in rapeseed (*Brassica napus* L.) seedlings. International Journal

Zhao, K., Fan, H., Zhou, S., & Song, J. (2003). Study on the salt and drought tolerance of *Suaeda salsa* and *Kalanchoe claigremontiana* under iso-osmotic salt and water stress. Plant Science, 165(4): 837-844.

Yanakieva, V., Parzhanova, A., Dimitrov, D., Vasileva, I., Raeva, P., Ivanova, S. (2023): Study on the antimicrobial activity of medicinal plant extracts and emulsion products with integrated herbal extracts. Agriculture and Forestry, 69 (4): 71-89. doi:10.17707/AgricultForest.69.4.06

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STUDY ON THE ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS AND EMULSION PRODUCTS WITH INTEGRATED HERBAL EXTRACTS

SUMMARY

The antimicrobial activity of water and oil extracts of five identified medicinal plants – thyme (*Thymus callieri* Borbás ex Velen), St. John's wort (*Hypericum perforatum* L.), cirsium (*Cirsium ligulare* Bois), hawthorn – flowers with leaf (*Crataegus monogyna* Jacq.), hawthorn–berries (*Crataegus monogyna* Jacq.) and juniper (*Juniperus communis* L) was investigated. The antimicrobial activity of the water extracts was tested against two groups of microorganisms: pathogenic (*Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Klebsiella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Candida albicans* NBIMCC 74 and *Listeria monocytogenes* ATCC 8632) and saprophytic (*Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Saccharomyces cerevisiae* ATCC 9763, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932). The obtained water extracts from all plant sources suppressed the growth of *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 8632, as well as the fungi *A. niger* ATCC 1015, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *F. moniliforme* ATCC 38932. The oil extracts showed inhibitory effect only on *S. aureus* ATCC 25923. In order to investigate their food biopreservation effect, the obtained plant extracts were

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incorporated in food matrix – mayonnaise, and stored for 20 days. During the storage, samples for microbiological analyses were taken. It was found that until the 12th day of the storage, in the controls as well as in the three samples of mayonnaise and the three samples of mayonnaise sauce, the yeasts and fungal counts were below the permissible values, while on the 20th day of the storage, visible fungal growth in all samples was observed.

Keywords: antimicrobial activity, pathogenic and saprophytic microorganisms, medicinal plants, mayonnaise, mayonnaise sauce

INTRODUCTION

Since ancient times, medicinal and flavoring plants and their essential oils have been known to have varying degrees of antimicrobial activity (Zaika, 1988). Historically, the products from natural sources, used in medicine, predate today's widespread antibiotic therapies and various medicinal drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief (Kafaru, 1994).

They are used as spices in the preparation of foods, improving their sensory profile and increasing their storage life. Spices or edible herbs have been shown to possess aromatic properties and exert antimicrobial effects on different pathogenic microorganisms (Brandi *et al*., 2006).

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis *et al*., 2006; Mc Lauchlin, 2004).

The antimicrobial and antioxidant properties of edible plants and the aromatic products obtained from them are due to substances with different chemical composition – essential and glyceride oils, alkaloids, flavonoids, tannins, glycosides and other compounds (Dobreva, 2010; Souza *et al.,* 2005; Suhaj, 2006; Obad *et al*., 2016; Teneva *et al*., 2016).

Even without a detailed understanding of the natural antimicrobial substances action, efforts to develop new effective methods aimed to improved the food safety are growing (Ayala Zavala *et al.,* 2008; Brandi *et al*., 2006; Lopez *et al*., 2007; Murdak *et al*., 2007; Nazef *et al*., 2008).

Thyme (*Thymus callieri* Borbás ex Velen) is a representative of the herbs with antimicrobial and antioxidant activity, which can be incorporated into food matrices preventing the growth of pathogenic and spoilage microorganisms and increasing their safety. However, the mechanisms of action, as well as the toxicological and sensory effects of the natural antimicrobial resources, are not fully understood (Burt, 2004; Davidson and Naidu, 2000; Gaysinsky and Weiss, 2007; Gutierrez *et al*., 2008b; Gutierrez *et al*., 2009; Lopez *et al*., 2008; Moriera *et al*., 2007; Patrignani *et al*., 2008; Periago *et al*., 2006; Ponce *et al.,* 2008). A number of studies consider the application of thyme extracts with proven antimicrobial activity and recommended its use as natural food preservatives (Bajpai *et al*., 2008; Burt, 2004; Sofia *et al.,* 2007). It has been reported that the thyme extracts added to meat products reduce the presence of pathogenic microorganisms (Grosso *et al*., 2008, Emiroğlu *et al*., 2010; Krishnan *et al.*, 2014).

Nowadays it is well known that the plants of the *Asteraceae* family have strong antimicrobial activity. The antimicrobial activity of *Achillea* spp. (Niño *et al.*, 2006; Paulo, 2006), *Arctotis auriculata* Jacq., and *Eriocephalus africanus* L. (Salie *et al*., 1996; Stojanovic *et al*., 2005) have been reported. Further, there are also many reports on the bioactivities, including antimicrobial activity, of extracts and essential oils from species of the genus Artemisia (Ramezani *et al*., 2004).

According to Tadic *et al.* (2008) extracts of dried hawthorn (*Crataegus monogyna* Jacq. and *Crataegus oxyacantha* L) can be used as an anti-inflammatory, gastroprotective and antimicrobial agent. Studies conducted by Kostić (2012), prove that hawthorn-berries extracts (*Crataegus oxyacantha* L.) are rich in polyphenolic compounds and exhibit good antioxidant and antimicrobial properties.

Rezvani and Mahmoodi (2009) demonstrated that the essential oils of *Juniperus communis* possessed antibacterial activity against *Staphylococcus aureus* NCIB 6751 and *Escherichia coli* NCIB 8879.

Food emulsions are the basis of many products, and a significant part of them are from the mayonnaise type, sweet and salty emulsion sauces and dressings. Nowadays, their composition includes not only glyceride oils, which are rich in polyunsaturated fatty acids (Nikovska 2008a, b; Nikovska and Stamov, 2009), but also oil extracts containing various biologically active substances (Boeva *et al*., 1990; Georgiev and Stoyanova, 2006).

The aim of the present study was to determine the antimicrobial activity of water and oil extracts from five types of medicinal plants against pathogenic and saprophytic microorganisms, as well as to investigate the possibility of their application in model formulations of various food products – mayonnaise, mayonnaise sauces and salad dressing.

MATERIAL AND METHODS

Materials

Culture media

<u>*Plate count agar*</u> (PCA). This medium was used for determination of the total plate count of mesophilic aerobic and facultative anaerobic microorganisms. A quantity of 23.5 g of the PCA agar medium base (containing 5 g casein peptone, 2.5 g yeast extract, 1 g dextrose and 15 g agar) was dissolved in 1 L of deionized water, pH 7.0±0.2. The medium was sterilized by autoclaving (regime:121 °C/15 min).

<u>*Chloramphenicol glucose agar (CGA*).</u> CGA is a selective medium for the enumeration of yeasts and fungi. A quantity of 40 g of the CGA agar medium base (containing 20 g dextrose, 5 g yeast extract, 0.1 g chloramphenicol and 15 g agar) was dissolved in 1 L of deionized water, pH 6.6±0.2. The medium was sterilized by autoclaving (regime: 121 °C/15 min).

Tryptone bile glucuronic agar (TBX). TBX is a selective medium for *Escherichia coli*. A quantity of 36.5 g of the TBX agar medium base (containing 20 g tryptone, 1.5 g bile salts, 0.075 g X-β-D-glucoronide and 15 g agar) was dissolved in 1 L of deionized water, pH 7.2±0.2. The medium was sterilized by autoclaving (regime: 121 °C/15 min).

Xylose Lysine Deoxycholate (XLD) Agar. XLD is a selective medium for *Salmonella*. A quantity of 55.2 g of the XLD agar medium base (containing lactose 7.5 g, sucrose 7.5 g, sodium thiosulfate 6.8 g, l-lysine 5.0 g, sodium chloride 5.0 g, xylose 3.75 g, yeast extract 3.0 g, sodium deoxycholate 2.5 g, ferric ammonium citrate 0.8 g, phenol red 0.08 g, agar 15.0) was dissolved in 1 L of deionized water, pH 7.4±0.2. The medium do not autoclave or overheat. After preparation the medium was directly transferred to water bath (50 °C).

Chapman agar (Mannitol salt agar). Chapman agar is a selective medium for *Staphylococcus aureus*. A quantity of 111 g of the Chapman agar medium base (containing 1 g beef extract, 5 g pancreatic digest of casein, 5 g peptic digest of meat, 75 g NaCl, 10 g D-mannitol, 0.025 g phenol red and 15 g agar) was dissolved in 1 L of deionized water, pH 7.4±0.2. The medium was sterilized by autoclaving (regime: 121 °C / 15 min).

Luria-Bertani agar medium supplemented with glucose (LBG agar). LBG agar was used for cultivation of test bacteria and implementation of antimicrobial activity assay. The LGB mixture contained following substances: tryptone (10 g), yeast extract (5 g), NaCl (10 g), glucose (10 g) and agar (15 g). The total quantity of 50 g was used. This mixture was dissolved in 1 L of deionized water, pH 7.5±0.2. The prepared medium was sterilized by autoclaving (regime: 121 °C/15 min).

Malt extract agar (MEA). Yeasts and fungi were cultivated in this medium. The MEA mixture contained following substances: malt extract (30 g), mycological peptone (5 g), agar (15 g). The total quantity of 50 g was used. This mixture was dissolved in 1 L of deionized water, pH 5.4±0.2. The medium was autoclaved at 115 °C for 10 min.

All culture media were prepared in accordance with the manufacturer's (Scharlab SL, Spain) instructions.

Plant sources

Five different Bulgarian medicinal plants that grow in the Western Rhodopes, Dospat municipality, Bulgaria were selected. The plants were collected during the period of their flowering, in sunny and dry weather – May-September 2019. To obtain the two types of extracts (water and oil) different morphological parts of the plants were used, namely: flowers of the cirsium (*Cirsium ligulare* Boiss) and hawthorn (*Crataegus monogyna*), flower-bearing stems – stalks with flowers of St. John's wort (*Hypericum perforatum* L.) and thyme (*Thymus callieri* Borbás ex Velen.). Hawthorn-berries (*Crataegus monogyna*) and juniper cones (*Juniperus communis* L) were also used, but they were harvested after the ripening of the berry and the cones – September-October

2019. The geographical coordinates from where herbs were harvested (presented in table 1) give the exact location of every single population on the map.

Table 1. Geographical coordinates of the herbs plantations

Herbs	Locality	Geographical coordinates	Altitude
Thyme	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63503 16655 Lat. 41.66583 Lon. 24.159444	UTM/MGRS KG61 1214 m
St. John's Wort	Bulgaria, Western Rhodopes, near the town of Dospat, Dulga Barchina locality	35TKG 64164 13506 Lat. 41.6377 Lon. 24.16861	UTM/MGRS KG61 1264 m
Cirsium	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63413 16751 Lat. 41.66666 Lon. 24.15833	UTM/MGRS KG61 1207 m
Hawthorn	Bulgaria, Blagoevgrad region, near the village of Satovcha, Aspen locality	35TKG 57029 12827 Lat. 41.62944 Lon. 24.50992	UTM/MGRS KG51 1134 m
Juniper cones	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63526 16655 Lat. 41.66583 Lon. 24.159722	UTM/MGRS KG61 1214 m

To obtain the extracts, dried plant materials were used. The fresh plant sources were identified, inspected to remove impurities and dried in a thin layer in the shade at a temperature of 22–25 °C. The dry plant mass was stored in paper, in well-closed bags, in a dry place until analysis.

Ingredients for the extracts and samples

For the preparation of water and oil extracts, experimental samples and control samples of mayonnaise, mayonnaise sauce and salad dressings, the following ingredients were used:

Main raw materials: vegetable oil (refined sunflower oil-oleic type), “Papas Oil” Ltd, Veliki Preslav, Bulgaria and water.

Additional and auxiliary raw materials: sugar (white, crystalline) – “Sweet Life”, Serbia; salt (cooking, table, iodized) –“Lubex” Ltd.; vinegar (5%, apple), “Veda” Ltd., Pleven, Bulgaria. The emulsifiers and stabilizers were provided by “Bobal-Boyadzhiev” Ltd., Sofia, Bulgaria. Emulsifiers and egg yolk substitutes – Trecomex Twelve – “Bobal-Boyadzhiev” Ltd., Sofia, Bulgaria and stabilizers –

Mayolys (mixture of vegetable gums) and Swely Gel Soft (modified starch, very strong thickening agent) – "Bobal-Boyadzhiev" Ltd., Sofia, Bulgaria.

Test microorganisms

For the determination of antimicrobial activity, the following test microorganisms from the National Bank for Industrial Microorganisms and Cell Cultures and the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria were used: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017, *Klebsiella sp.* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 8632, *Listeria monocytogenes* NCTC 11994, *Candida albicans* NBIMCC 74, *Saccharomyces cerevisiae* ATCC 9763, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932.

The strains were cultivated as follows: *Saccharomyces cerevisiae* ATCC 9763 cultivated at 30°C on Malt extract agar, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932 were cultivated at 30°C on LBG-agar, and *Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Klebsiella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Candida albicans* NBIMCC 74, *Listeria monocytogenes* ATCC 8632, *Listeria monocytogenes* NCTC 11994, *Salmonella enterica* subsp. enterica serovar abony NCTC 6017 – at 37 °C on LBG-agar.

Methods

Preparation of oil extracts

The extraction procedure was carried out according to the methods of Stoyanova (1986) and Georgiev and Stoyanova (2006). To obtain oil extracts, pre-ground and moistened to a 70% humidity (Georgiev, 1998) dry plant raw materials from the studied plant species were used and they were subjected to extraction by soaking for 24÷48 hours at 80 °C and with continuous stirring.

Preparation of water extracts

To obtain decoction-type water extracts, a hydromodule of 1:20 was maintained. In our case, 15 g of the raw material was weighed and transferred to a flask containing 300 cm^3 of extractant – water heated to 60–65 °C. The temperature was maintained for 1 h, after which the mixture was filtered through kapron cloth. The residue was returned to the flask and poured with 200 cm^3 of the appropriate extractant (water). The second extraction lasted 1 hour, at the same temperature (60–65 °C), and then filtered. The two filtrates were combined and homogenized well.

Determination of oil extracts antimicrobial activity

The antimicrobial activity of the oil extracts was determined by a modification of the "diffusion in agar" method, by measuring the inhibition zones of the pathogens growth around metal rings in which a certain amount of oil extract was introduced. The selective media for *Listeria monocytogenes* NCTC 11994, *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017 and *Staphylococcus aureus* ATCC 25923 were inoculated with suspensions of the pathogens prepared from a 24 hour culture on slanted PCA. From a suitable ten fold suspension dilution, the melted and cooled to 45–50 °C selective media were inoculated. The effective concentration of the cells in the agar was equated to the concentration of the 1.0×105 dilution suspension, as 1 cm^3 of suspension was inoculated into 99 cm^3 of medium. After solidification of the media, sterilized metal rings (Ø=6 mm) were placed on their surface, in which 0.05; 0.10 and 0.15 cm^3 extract was added. Petri dishes were incubated at 37 °C. The diameter [mm] of the growth inhibition zones of tested microorganisms at 24 and 48 hours was measured and a comparative assessment of their antibacterial activity was made.

Determination of water extracts antimicrobial activity

The antimicrobial activity of water extracts was determined by the agar-well diffusion method, while the minimum inhibitory concentration (MIC) was determined by the serial dilution method (Jirovetz *et al.*, 2006).

Agar-well diffusion method. In Petri dishes (d=9 cm), placed on a level surface, 17 ml of pre-melted, cooled to 40–45 °C and inoculated with the test microorganism (1.0×10^6 cfu/cm^3 for fungal spore sand 1.0×10^8 cfu/cm^3 for viable bacterial and yeast cells) LBG-agar medium was poured. After spilling of the inoculated culture media, the Petri dishes were left for 1 hour to solidify the agar. Next, six wells per dish (d=6 mm) were cut, and 60 μl of each water extract were pipetted into the agar wells, in duplicate. The Petri dishes were thermostated at different temperature conditions (depending on the type of test microorganism) for 24÷48 hours. The presence and degree of antimicrobial activity was evaluated by the formed inhibition zones (IZ, mm) around the wells.

Minimum inhibitory concentration (MIC) by serial dilution method: The minimum inhibitory concentration (MIC) of the water extracts was determined in order to calculate their amount for application into food raw materials and products as a biopreservative. For this purpose, the water extracts were diluted twice in 0.9% NaCl. Then, a test for antimicrobial activity was carried out, determining the minimum inhibitory concentration – MIC (the highest dilution of the water extracts which inhibited the growth of the test microorganism around the agar wells).

Preparation of mayonnaise and mayonnaise sauce

The technologies for mayonnaise and dressing-type mayonnaise sauce production was proposed by Perifanova-Nemska and Uzunova (2016). Based on preliminary research on food O/W emulsions with incorporated extracts of the investigated plant materials, three assortments remained as a result of the selection, on which the research was continued. The developed formulation presented in table 2.

Table 2. Model recipe composition of mayonnaise, mayonnaise sauce and salad dressing

Raw materials, %	Assortments					
	Mayonnaise		Mayonnaise sauce		Salad dressing	
	Control Sample	Experimental Samples	Control Sample	Experimental Samples	Control Sample	Experimental Samples
Vegetable Oil	50.00	–	25.00	–	56.00	–
Oil extracts	–	50.00	–	25.00	–	56.00
Mayolys SX	0.30	0.30	0.15	0.15	–	–
Sweelygel	1.50	1.50	0.75	0.75	–	–
Water	45.70	45.70	23.35	23.35	20.00	–
Water extract	–	–	48.00	48.00	–	20.00
Vinegar	–	–	–	–	20.00	20.00
Trecomex	0.50	0.50	0.75	0.75	–	–
Salt	1.00	1.00	1.00	1.00	3.00	3.00
Sugar	1.00	1.00	1.00	1.00	1.00	1.00

All three culinary products were prepared using oil extracts for the oil phase of the assortments. Water extracts were included in the recipe compositions of the mayonnaise sauce and the salad dressings. The six samples were compared with control samples in which refined high oleic sunflower oil and water were included.

Organoleptic analysis of food emulsion products

To conduct the organoleptic analysis, 20 unbiased testers over 18 years old were invited. The sensory evaluation was performed using a five-point hedonic scale, where 5 corresponded to the highest and 1 to the lowest evaluation for the given indicator. The samples were evaluated according to the following quality indicators: appearance, consistency, color, smell, aroma, taste, aftertaste. Based on the conducted tasting, a general perception assessment of the final products was formed.

Microbiological analysis

The total plate count (cfu/g) (mesophilic aerobic and facultative anaerobic microorganisms), yeast and fungal counts (cfu/g), and presence of specific pathogenic microorganisms (cfu/g) were determined according to the Bulgarian State Standards (table 3).

Table 3. Microbiological parameters and standards for foods

Parameter	Culture medium / t°C	Standard
Total plate count*	PCA / 30°C	BSS EN ISO 4833-1, 2013
Yeasts and fungi*	CGA / 30°C	BSS EN ISO 21527-2, 2011
*Escherichia coli**	TBX agar / 44°C	BSS EN ISO 16649-2, 2014
Salmonella sp.**	XLD agar / 37°C	BSS EN ISO 6579-1, 2017
*Staphylococcus aureus***	Chapman agar / 37°C	BSS EN ISO 6888-1, 2005

* *–pour-plating method; ** –spread-plating method*

RESULTS AND DISCUSSION

The concentrations of pathogenic and saprophytic microorganisms are presented in table 4.

Table 4. Concentrations of pathogenic and saprophytic test microorganisms

Pathogenic test-microorganism	Concentration, cfu/cm^3
Salmonella enteritidis ATCC 13076	2.0×10^8
Klebsiella sp. (clinical isolate)	2.7×10^8
Escherichia coli ATCC 8739	1.0×10^{12}
Candida albicans NBIMCC 74	2.0×10^{10}
Listeria monocytogenes ATCC 8632	4.6×10^9
Staphylococcus aureus ATCC 25923	4.0×10^8
Salmonella enterica subsp. *enterica* serovar *abony* NCTC 6017	3.5×10^8
Listeria monocytogenes NCTC 11994	7.0×10^8
Saprophytic test-microorganism	Concentration, cfu/cm^3
Bacillus subtilis ATCC 6633	1.0×10^9
Bacillus cereus ATCC 11778	6.5×10^8
Saccharomyces cerevisiae ATCC 9763	2.8×10^7
Aspergillus niger ATCC 1015	4.0×10^8
Aspergillus flavus	4.0×10^8
Penicillium sp.	6.0×10^8
Rhizopus sp.	1.2×10^7
Fusarium moniliforme ATCC 38932	1.2×10^7

Table 5. Antimicrobial activity of oil extracts against pathogenic microorganisms

Oil extracts	Concentration, cm^3	*Escherichia coli* ATCC 8739	*Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017	*Listeria monocytogenes* NCTC 11994	*Staphylococcus aureus* ATCC 25923
Thyme *Thymus callieri* Borbás ex Velen.	0.15	–*	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–
St. John'sWort *Hypericum perforatum* L.	0.15	–	–	–	15
	0.10	–	–	–	–
	0.05	–	–	–	–
Cirsium *Cirsium ligulare* Boiss.	0.15	–	–	–	–
	0.10	–	–	–	–
	70.05	–	–	–	–
Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.	0.15	–	–	–	14
	0.10	–	–	–	12
	0.05	–	–	–	11
Hawthorn /berries/ *Crataegus monogyna* Jacq.	0.15	–	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–
Juniper *Juniperus communis* L.	0.15	–	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–

– diameter of the zone equal to the diameter of the metal ring is taken as a negative result*

Oil extracts antimicrobial activity

The data from the antimicrobial activity (table 5) of oil extracts indicated that the extracts from thyme, St. John's wort, cirsium, hawthorn (flowers or berries), and juniper had weak antimicrobial activity against pathogenic microorganisms. The tested extracts showed inhibitory activity only against the Gram-positive *Staphylococcus aureus* ATCC 25923. The obtained results showed that *Staphylococcus aureus* ATCC 25923 was more sensitive to the hawthorn (flowers) extract at all three concentrations (0.15; 0.10 and 0.05 cm^3), with inhibition zones: 14, 12 and 11 mm, respectively. For an oil extract obtained from St. John's wort, an inhibition zone of 15 mm was found for the extract concentration of 0.15 cm^3.

Water extracts antimicrobial activity

The inhibition zone (IZ) and the minimum inhibitory concentration (MIC) were determined.

The results from the determination of antimicrobial effect of the water extracts are presented in table 6 and table 7. It was found that all six extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium did not influence the growth of the test microorganisms – the spore-forming *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 11778, and the yeasts *Saccharomyces cerevisiae* ATCC 9763.

The experimental data indicated that water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium, suppressed the growth of the fungi *Aspergillus niger* ATCC 1015 and *Penicillium* sp., in which was established the inhibition zones from 8 to 13 mm and MIC in the range of 60 to 600 ppm. The highest inhibitory activity against *Rhizopus* sp. was found in the thyme extract with inhibition zones of 14 mm and MIC<60 ppm, followed by the extracts of hawthorn (berries), juniper, cirsium and hawthorn (flowers), where the zones of inhibition were between 8 and 12 mm and MIC was 60 ppm. In the water extract of St. John's wort, antimicrobial effect against *Rhizopus* sp. was not observed.

Regarding the fungi *Aspergillus flavus* and *Fusarium moniliforme* ATCC 38932, an inhibitory effect was found only from the extracts of thyme and cirsium.

The data in table 7 indicated that water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium had weak antimicrobial activity against pathogenic microorganisms. Inhibitory activity only against Gram-positive *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 8632 was found.

A higher antimicrobial effect was found against *Staphylococcus aureus* ATCC 25923 with inhibition zones between 10 to 13 mm and MIC of 600 ppm. The obtained results showed that *Listeria monocytogenes* ATCC 8632 was more sensitive to the extract of hawthorn (berries). The inhibition zones were up to 10.5 mm, while the MIC value was 60 ppm.

The Gram-positive bacteria were more sensitive to the tested extracts (inhibition zones between 10.5 to 13.5 mm), with MIC of 600 ppm. The tested Gram-negative bacteria were less sensitive and inhibitory effect was not found. This is due to the difference in the structure and composition of the cell wall of the two groups bacteria. The presence of an outer membrane in Gram-negative bacteria makes it difficult for the extracts to diffuse through the membrane to the cell cytoplasm, which makes them more resistant to the action of the studied extracts. The obtained results on the different resistance of Gram-positive and Gram-negative bacteria to microbial growth inhibitors were in correlation with the literature data on aromatic products from the studied herbs (Randrianarivelo *et al*., 2009; Souza *et al*., 2005; Teneva *et al*., 2015).

Krtivokapić *et al*. (2021) found, when studying the phenolic composition of *Hypericum perforatum* L and *Melissa officinalis* L from Montenegro, that the type of solvent used and the altitude where the herbs grown had a significant influence on the composition of the extracted substances. This was also consistent with the established different antimicrobial activity found in the different extracts in our study. The composition of the herbs, which determined their antimicrobial activity was extremely influenced by the climate of the area where the plants grown. In this line of thought, Myrtaj *et al*. (2022) demonstrated that climate influenced the essential oil composition of *Salvia officinalis* in 4 populations of the plant grown in a mountainous region of Southern Albania. The importance of the effects of various extracts from plant sources is increasing in view of their broad-spectrum biological activities, part of which is the antimicrobial effect (Petrović *et al.,* 2022).

Organoleptic analysis

Figures 1 and 2 present the results from the organoleptic analysis of mayonnaise with added oil extracts and mayonnaise sauce with added oil and water extracts. From the data, it could be seen that in terms of appearance and color, mayonnaise with added St. John's wort oil extract was the most prefered, followed by that with added thyme oil extract. While in the case of mayonnaise sauces, sample 3 (cirsium) and 4 (hawthorn-flowers) were the best perceived, and with the most liked color was the sauces with integrated hawthorn extracts (flowers and berries). According to evaluators, the dressings had a light, almost white color, which was due to the low volume of the oil phase. Other authors also confirm the light color of low-fat emulsion products, which, according to them, was also due to the presence of water-phase thickening substances – starch and gums (Karas *et al.,* 2002).

The assortments were with the same consistency and stability. There was no delamination and separation of oil on their surface. In terms of viscosity, the evaluators defined the dressings as products with a rather liquid consistency, which in the case of mayonnaise was spreadable.

With the most intense smell, the evaluators defined mayonnaise containing oil extract of thyme, followed by the same one with St. John's wort and hawthorn-berries. This was also the reason why this assortment was accepted even with the most intense taste. The salty taste as an indicator was not commented by the evaluators, since the amount of salt was the same for all assortments. The evaluators did not report a rancid taste and smell in the products, which was an indicator of the quality of the vegetable oil used.

After consumption, the aftertaste of thyme and hawthorn-berries was more pronounced, which was rated with the highest intensity of perception. The aftertaste of juniper was bitter and unpleasant, followed by St. John's Wort.

From the results of the tasting analysis of the salad dressing, which are presented graphically in figure 3 it was evident that the same trend was maintained. For all indicators, the dressing with thyme received the highest score, followed by the dressing with cirsium and hawthorn-berries. The lowest scores were obtained for the indicators of St. John's wort and juniper.

The results of the general sensory evaluations of the mayonnaise, mayonnaise sauce and salad dressing assortments are placed in table 8.

Table 6. Antimicrobial activity and minimum inhibitory concentration (MIC) of water extracts against saprophytic microorganisms

Saprophytic microorganisms	Thyme *Thymus callieri* Borbás ex Velen.		St. John'sWort *Hypericum perforatum* L.		Cirsium *Cirsium ligulare* Boiss.		Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.		Hawthorn /berries/ *Crataegus monogyna* Jacq.		Juniper *Juniperus communis* L.	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Bacillus subtilis ATCC 6633, 1.0.10^{9} cfu/cm^{3}	–*	–	–	–	–	–	–	–	–	–	–	–
Bacillus cereus ATCC 11778, 6.5.10^{8} cfu/cm^{3}	–	–	–	–	–	–	–	–	–	–	–	–
Saccharomyces cerevisiae ATCC 9763, 2.8.10^{7} cfu/cm^{3}	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus niger ATCC 1015, 4.0.10^{8} cfu/cm^{3}	13.00 ±1.00	<60	12.00 ±0.00	<600	12.50 ±0.60	<60	10.00 ±0.00	60	10.00 ±0.00	60	8.00 ±0.00	60
Aspergillus flavus, 4.0.10^{8} cfu/cm^{3}	10.00 ±0.00	60	–	–	11.00 ±0.00	<600	–	–	–	–	–	–
Penicillium sp., 6.0.10^{8} cfu/cm^{3}	10.00 ±0.00	60	10.00 ±0.00	60	10.00 ±0.00	60	12.00 ±0.00	<600	12.00 ±0.00	<600	10.00 ±0.00	60
*Rhizopus*sp., 1.2.10^{7} cfu/cm^{3}	14.00 ±0.40	<60	–	–	10.00 ±2.00	60	8.00 ±0.00	60	12.00 ±0.00	<60	11.00 ±0.00	<60
Fusarium moniliforme ATCC 38932, 1.2.10^{7} cfu/cm^{3}	8.00 ±0.00	60	–	–	8.00 ±0.00	600	–	–	–	–	–	–

–* *not detected*

Table 7. Antimicrobial activity and minimum inhibitory concentration (MIC) of water extracts against pathogenic microorganisms

Pathogenic microorganisms	Thyme *Thymus callieri* Borbás ex Velen.		St. John'sWort *Hypericum perforatum* L.		Cirsium *Cirsium ligulare* Boiss.		Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.		Hawthorn /berries/ *Crataegus monogyna* Jacq.		Juniper *Juniperus communis* L.	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Salmonella enteritidis ATCC 13076, 2.0. 10^{8} cfu/cm^{3}	–*	–	–	–	–	–	–	–	–	–	–	–
Klebsiella sp. (clinical isolate), 2.7. 10^{8} cfu/cm^{3}	–	–	–	–	–	–	–	–	–	–	–	–
Escherichia coli ATCC 8739, 1.0. 10^{12} cfu/cm^{3}	–	–	–	–	–	–	–	–	–	–	–	–
Candida albicans NBIMCC 74, 2.0. 10^{10} cfu/cm^{3}	–	–	–	–	–	–	–	–	–	–	–	–
Listeria monocytogenes ATCC 8632,4.6. 10^{9} cfu/cm^{3}	–	–	–	–	–	–	–	–	10.50 ±0.60	60	–	–
Staphylococcus aureus ATCC 25923, 4.0. 10^{8} cfu/cm^{3}	13.00 ±1.00	600	10.00 ±1.00	600	10.00 ±1.00	600	13.00 ±1.00	<600	13.50 ±2.00	<600	11.50 ±2.00	<600

–* *not detected*

The results of the individual indicators and those of the general perception of mayonnaise showed that the evaluators perceived the best mayonnaise with integrated oil extracts obtained from thyme, St. John's wort and hawthorn-berries. They corresponded to samples 1, 2 and 5.

A higher general evaluation compared to the other assortments was given to mayonnaise sauce dressing type with added oil and water extracts obtained from thyme, cirsium and hawthorn-berries, samples 1, 3 and 5, respectively. Consumers did not perceive dressings with added extracts of St. John's wort and juniper berries.

Based on the obtained results, three samples of mayonnaise were selected, namely variants 1, 2 and 5, which corresponded to mayonnaise with added extracts of medicinal plants – thyme, St. John's wort and hawthorn-berries.

From the six examined samples of mayonnaise sauce, variants 1, 3 and 5 were selected, respectively with added extracts of thyme, cirsium and hawthorn-berries.

Microbiological analysis – microbial contamination of mayonnaise and mayonnaise sauce

Microbiological analysis on day 1, day 4, day 8, day 12 and day 20 of three mayonnaise variants (variants 1, 2 and 5) and three mayonnaise sauce variants (variants 1, 3 and 5), compared with corresponding control samples was performed. The results demonstrated that during the storage, the presence of *Escherichia coli* and coagulase-positive staphylococci was below the acceptable limits. *Salmonella* sp. in the two control samples and in the three variants of mayonnaise and mayonnaise sauce during the entire storage period was not detected.

The experimental results presented in table 9 showed that mayonnaise (variant 2), prepared with St. John's wort, had a longer shelf life compared to variant 1 (thyme) and variant 5 (hawthorn-berries) mayonnaises, while in the other two variants, after the 8^{th} day, an increase in microbial insemination above the permissible values was observed.

From the results showed in table 10 for mayonnaise sauce, it was found that variant 1 (thyme) had a lower microbial population, which on the 12^{th} day reached 1×10^4 CFU/g, which was in accordance with the permissible values compared to the standard requirements. For mayonnaise sauce variant 3 (cirsium) and variant 5 (hawthorn-berries) on the 12^{th} day, the total plate count (mesophilic aerobic and facultatively anaerobic microorganisms) reached value of 1×10^5 CFU/g, therefore, they had a certain shelf life until the 8^{th} day.

It was found that until the 12^{th} day of the storage, in the controls as well as in the three samples of mayonnaise and the three samples of mayonnaise sauce, the yeasts and fungal counts were below the permissible values, while on the 20^{th} day of the storage, visible fungal growth in all samples was observed.

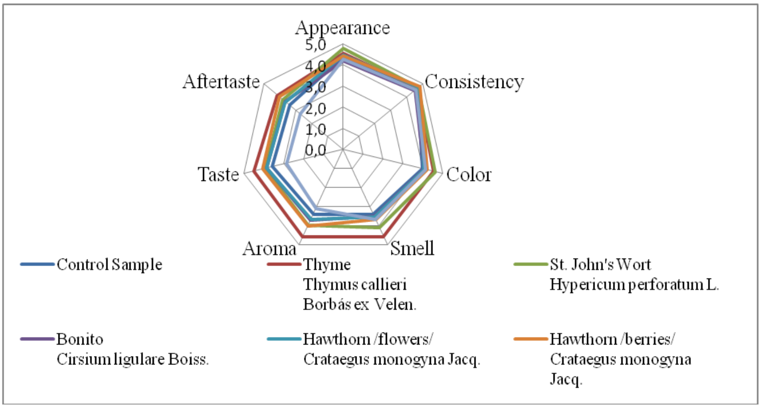

Figure 1. Tasting analysis results of mayonnaise with integrated oil extracts

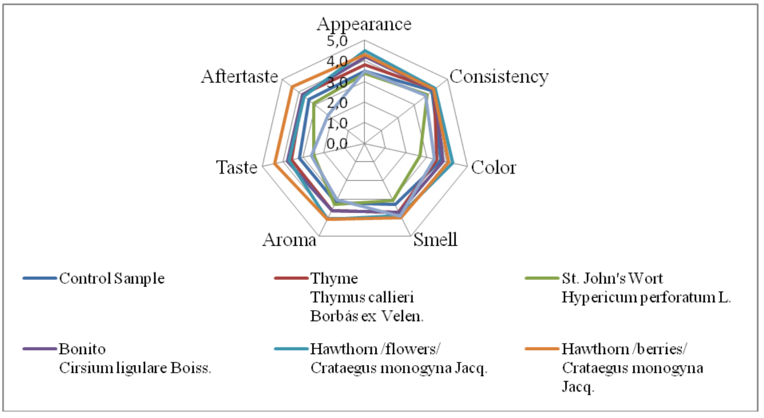

Figure 2. Tasting analysis results of mayonnaise sauce dressing type with integrated oil and water extracts

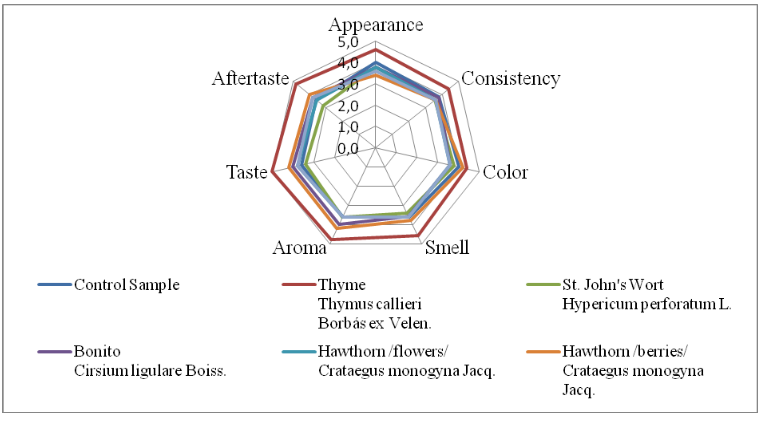

Figure 3. Tasting analysis results of the of salad dressing with oil and water extracts

Table 8. Data on the overall sensory evaluation of the three assortments

General sensory evaluation	Control Sample	Thyme *Thymus callieri Borbás exVelen*	St. John's Wort *Hypericum perforatum* L.	Cirsium *Cirsium ligulare* Boiss.	Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.	Hawthorn /berries/ *Crataegus monogyna* Jacq.	Juniper *Juniperus communis* L.
		Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6
Mayonnaise	26.9	31.6	29.9	27.5	28.0	29.1	25.3
Mayonnaise sauce	24.4	26.2	22.3	28.5	27.1	29.5	21.9
Salad dressing	26.2	32.6	25.6	26.6	25.6	27.4	24.8

Table 9. Microbiological indicators during storage of mayonnaise (for 20 days) at a temperature of 4 – 6 °C

Sample	Control					Variant 1 Thyme					Variant 2 St. John'sWort					Variant 5 Hawthorn /berries/				
Day	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20
Total count of mesophilic aerobic and facultative anaerobic bacteria, CFU/g	1×10^3	1×10^4	2.5×10^4	2.5×10^4	Visible mold	1.1×10^2	8×10^2	1×10^3	1×10^5	Visible mold	1.0×10^2	1.3×10^2	1.6×10^3	1.6×10^4	Visible mold	1.1×10^2	1.2×10^2	7.5×10^2	1.3×10^5	Visible mold
Yests, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	1×10^2		<10	<10	<10	1×10^2	
Fungi, CFU/g	10	20	50	50		<10	<10	10	50		<10	10	10	10		<10	10	20	1×10^2	
Escherichia coli, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	<10	
Staphylococcus aureus, CFU/g	<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100	
Salmonella, CFU/g	–*	–	–	–		–	–	–	–		–	–	–	–		–	–	–	–	

–* *not detected*

Table 10. Microbiological indicators during storage of mayonnaise sauce (for 20 days) at a temperature of 4 – 6 °C

Sample	Control					Variant 1 Thyme					Variant 3 Cirsium					Variant 5 Hawthorn /berries/				
Day	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20
Total count of mesophilic aerobic and facultative anaerobic bacteria, CFU/g	7×10^3	1×10^4	4×10^4	7.5×10^4	Visible mold	6.8×10^2	1.3×10^3	9.5×10^3	1×10^4	Visible mold	1×10^3	1×10^4	3×10^4	1×10^5	Visible mold	8×10^3	1.5×10^4	4×10^4	1×10^5	Visible mold
Yests, CFU/g	<10	<10	<10	<10		1.1×10^2	1.2×10^2	1.2×10^2	1.5×10^2		1×10^2	1.1×10^2	1.2×10^2	1.4×10^2		1.0×10^2	1.2×10^2	1.5×10^2	1.8×10^2	
Fungi, CFU/g	10	50	50	20		<10	20	10	20		<10	10	1×10^2	1.5×10^2		<10	<10	10	1×10^2	
Escherichia coli, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	4×10^4	<10	<10		<10	<10	<10	<10	
Staphylococcus aureus, CFU/g	<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100	
Salmonella, CFU/g	–*	–	–	–		–	–	–	–		–	–	–	–		–	–	–	–	

–* *not detected*

CONCLUSIONS

The following conclusions could be made from the studies carried out:

• The oil extracts from the investigated plant sources exhibited weak antimicrobial activity against the used pathogens. This type of extracts showed inhibitory activity only against the pathogen *Staphylococcus aureus* ATCC 25923.

• The water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium suppressed the growth of pathogens *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 8632, and fungi *A. niger* ATCC 1015, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *F. moniliforme* ATCC 38932 (agents of microbial spoilage).

• The added oil extracts from the medicinal plants – thyme, St. John's wort and hawthorn improved the organoleptic characteristics of the mayonnaise. The same trend was observed in mayonnaise sauce with added extracts of thyme, cirsium and hawthorn-berries.

• The microbiological research and analysis, necessary for the technological production, safety and control, of the three mayonnaises and three mayonnaise sauces rated the highest by the tasters, and compared with the control samples, were made.

• The obtained results of the present study can be used as a basis for developing of technologies related with obtaining of different groups emulsion products with the addition of extracts from plant sources acting as biopreservatives.

REFERENCES

Ayala Zavala, J., Del Toro Sánchez, L., Álvarez Parrilla, E. & González Aguilar, G. (2008). High relative humidity in package of fresh cut fruits and vegetables: advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? Journal of Food Science, 73(4): R41–R47.

Bajpai, V. K., Rahman, A. & Kang, S. C. (2008). Chemical composition and inhibitory parameters of essential oil and extracts of *Nandina domestica* Thunb. to control food-borne pathogenic and spoilage bacteria. International Journal of Food Microbiology, 125(2): 117–122.

Brandi, G., Amagliani, G., Schiavano, G. F., De Santi, M. & Sisti, M. (2006). Activity of *Brassicaoleracea* leaf juice on food borne pathogenic bacteria. Journal of Food Protection, 69(9): 2274–2279.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. International Journal of Food Microbiology, 94(3): 223–253.

Davidson, P.M. & Naidu, A.S. (2000). Phytophenols. In A. S. Naidu (Ed.), Natural food antimicrobial systems (pp. 265–295). Boca Raton, Florida: CRC Press.

Dobreva, A., Tintchev, F., Heinz, V., Schulz, H. & Toepfl, S. (2010). Effect of pulsed electric fields (PEF) on oil yield and quality during distillation of white oil-bearing rose (Rosa alba L.). Zeitschriftfür Arznei and Gewürzpflanzen, 15(3):127–132.

Emiroğlu, Z., Yemiş, G., Coşkun, B. & Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Science, 86: 283–288.

Gaysinsky, S. & Weiss, J. (2007). Aromatic and spice plants: Uses in food safety. Stewart Postharvest Review, 4(5): 1–9.

Georgiev, E. (1998). Technology of natural and synthetic aromatic products, Plovdiv.

Georgiev, E. & Stoyanova, A. (2006). Manual of the specialist in the aromatic industry, Plovdiv, BNAEMPC.

Grosso, C., Ferraro, V., Figueiredo, A. C., Barroso, J. G., Coelho, J. A. & Palavra, A. M. (2008). Super critical carbon dioxide extraction of volatile oil from Italian coriander seeds. Food Chemistry, 111(1): 197–203.

Gutierrez, J., Barry-Ryan, C. & Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: Efficancy, synergistic potential and interactions with food components. Food Microbiology, 26: 142–150.

Gutierrez, J., Rodriguez, G., Barry-Ryan, C. & Bourke, P. (2008b). Efficacy of plant essential oils against food borne pathogens and spoilage bacteria associated with ready-to-eat vegetables: Antimicrobial and sensory screening. Journal of Food Protection, 71(9): 1846–1854.

Jirovetz, L., Buchbauer, G., Denkova, Z., Slavchev, A., Stoyanova, A. & Schmidt, E. (2006). Chemical composition, antimicrobial activities and odor descriptions of various *Salvia* sp. and *Thuja* sp. essential oils. Ernahrung/Nutrition, 30: 152–159.

Kafaru, E. (1994). Immense help formative workshop. In Essential Pharmacology, 1st Ed. ed; Elizabeth Kafaru Publishers: Lagos, Nigeria, 1994.

Kostić, D., Velicković, J., Mitić, S., Milan, M. & Randelović, S. (2012). Phenolic content, and antioxidant and antimicrobial activities of *Crataegus oxyacantha* L (*Rosaceae*) fruit extract from South East Serbia. Tropical Journal of Pharmaceutical Research, 11 (1): 117-124.

Krishnan, R., Babuskin, P., Babu, A., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M. & Sukumara, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. International Journal of Food Microbiology, 171: 32–40.

Krivokapić, S., Pejatović, T. (2021). Effect of the vegetation cycle on total phenolic and flavonoid compounds in *Hypericum perforatum* L. and *Melissa officinalis* L. collected in Montenegro. Agriculture and Forestry, 67(1): 181-190.

Lewis, K. & Ausubel, F.M. (2006). Prospects of plant derived antibacterials. Nature Biotechnology, 24: 1504–1507.

Lopez, M. D., Jordan, M. J. & Pascual-Villalobos, M. J. (2008). Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. Journal of Stored Products Research, 44(3): 273–278.

Lopez, P., Sanchez, C., Battle, R. & Nerian, C. (2007). Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against food borne microorganisms. Journal of Agricultural and Food Chemistry, 55(11): 4348–4356.

Mc Lauchlin, J., Mitchell, R.T., Smerdon, W.J. & Jewell, K. (2004). *Listeria monocytogenes* and *listeriosis*: a review of hazard characterisation for use in microbiological risk assessment of foods. International Journal of Food Microbiology, 92(1): 15-33.

Moriera, M. R., Ponce, A. G., Del Valle, C. E. & Roura, S. (2007). Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanching spinach and minced cooked beef. Journal of Food Processing and Preservation, 31: 379–391.

Murdak, C. A., Cleveland, J., Matthews, K. R. & Chikindas, M. L. (2007). The synergistic effect of nisin and lactoferrin on the inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7. Letters in Applied Microbiology, 44: 255 – 261.

Myrtaj, B., Dervishi, A., Nuro, A., Salihila, J., Peci, D. (2022). Climate influence and essential oils composition of *Salvia officinalis* in populations of Southern Albania. Agriculture and Forestry, 68(4): 123-134.

Nazef, L., Belguesmia, Y., Tani, A., Prevost, H. & Drider, D. (2008). Identification of lactic acid bacteria from poultry feces: Evidence on anti-campylobacter and anti-listeria activities. Poultry Science, 87(2): 329–334.

Niño, J, Narváez, D.M., Mosquera, O.M. & Correa, Y.M. (2006). Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two *Rubiaceae* plants from Colombian biodiversity. Brazilian Journal of Microbiology, 37(4): 566-570.

Obad, K., Khaled, S.A., Abdulla, M.L., Bekshokov, K.S. & Saidov, M.S. (2016). Antimicrobial activity of some essential oils against *Staphyloccocus aureus* and *E. Coli*. World Journal of Pharmaceutical Research, 5(4): 1774 – 1779.

Patrignani, F., Iucci, L., Belletti, N., Gardini, F., Guerzoni, M. E. & Lanciotti, R. (2008). Effects of sub-lethal concentrations of hexanal and 2-(E)-hexenal on membrane fatty acid composition and volatile compounds of *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella enteritidis* and *Escherichia coli*. International Journalof Food Microbiology, 123(1–3): 1–8.

Paulo, S. (2006). Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from columbian biodiversity. Brazilian Journal of Microbiology, 37(4): 321-35.

Periago, P. M., Conesa, R., Delgado, B., Fernandez, P. S. & Palop, A. (2006). *Bacillus megaterium* spore germination and growth inhibition by a treatment combining heat with natural antimicrobials. Food Technology and Biotechnology, 44(1): 17–23.

Ponce, A. G., Roura, S. I., Del Valle, C. E. & Moreira, M. R. (2008). Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: In vitro and in vivo studies. Postharvest Biology and Technology, 49(2): 294–300.

Petrović, B., Vukomanović, P., Popović, V., Šarčević-Todosijević, Lj., Burić, M., Nikolić, M., Đorđević, S. (2022). Significance and efficacy of triterpene saponin herbal drugs with expectorant action in cough therapy. Agriculture and Forestry, 68(3): 221-239.

Ramezani, M, Fazli-Bazzaz, B.S, Saghafi-Khadem, F., Dabaghian, A. (2004). Antimicrobial activity of four Artemisia species of Iran. Fitoterapia, 75(2): 201-203.

Randrianarivelo, R., Sarter, C., Odoux, E., Brat, P., Lebrun, M., Romestand, B., Menut, C., Andrianoelisoa, H.S., Raherimandimby, M. & Danthu, P. (2009). Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. Food Chemistry, 114: 680–684.

Rezvani, S., Rezai, M.A., & Mahmoodi, N. (2009). Analysis and antimicrobial activity of the plant, *Juniperus communis*. Rasayan Journal of Chemistry, 2(1): 257-260.

Salie, F, Eagles, P.F. & Leng, H.M. (1996). Preliminary antimicrobial screening of four South African *Asteraceae* species. Journal of Ethnopharmacology, 52(1): 27-33.

Sofia, P. K., Prasad, R., Vijay, V.K. & Srivastava, A.K. (2007). Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. International Journalof Food Science and Technology, 42: 910–915.

Souza, E., Stamford, T., Lima, E., Trajano, V. & Filho, J. (2005). Antimicrobial effectiveness of spices: an approach for use in food conservation systems. Brazilian Archives of Biology and Technology, 48: 549–558.

Stojanovic, G., Radulovic, N., Hashimoto, T. & Palic, R. (2005). In vitro antimicrobial activity of extracts of four Achillea species: the composition of *Achillea clavennae* L. (*Asteraceae*) extract. Journal of Ethnopharmacology, 101(1-3): 185-90.

Stoyanova, A. (1986). Study on the Production Technology of St. John's Wort (*Hypericum perforatum* L) Extracts for Cosmetic Purposes". Dissertation for obtaining a scientific degree "candidate of technical sciences", UFT, Plovdiv.

Suhaj, M. (2006). Spice antioxidants isolation and their antiradical activity. Journal of Food Composition and Analysis, 19: 531–537.

Tadic, M.V., Dobric, S., Markovic, M.G., Đordjevic, M.S. & Arsic, A.I., (2008). Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of Hawthorn berries ethanol extract. Journal of Agricultural and Food Chemistry, 56: 7700-7709.

Teneva, D., Denkova, Z., Denkova, R., Atanasova, T., Nenov, N. & Merdzhanov, P. (2015). Antimicrobial activity of essential oils and extracts from black pepper, cumin, coriander and cardamom. Bulgarian Journal of Veterinary Medicine, 18(4): 373 – 377.

Teneva, D., Denkova, Z., Goranov, B., Denkova, R., Kostov, G., Atanasova, T. & Merdzhanov, P. (2016). Chemical composition and antimicrobial activity of essential oils from black pepper, cumin, coriander and cardamom against some pathogenic microorganisms. Acta Universitatis Cabiniensis Series E: Food Technology, XX (2): 39 – 52.

Zaika, L.L. (1988). Spices and herbs: their antimicrobial activity and its determination. Journal of Food Safety, 9(2): 97–118.

Amraoui, M., Mijanovic, D., El Amrani, M., Kader, S. and Ouakhir, H. (2023). Agriculture and economic development of the Ait Werra tribe during the French colonialism period and its local characteristics (1912-1956) within the middle atlas region of Morocco. Agriculture and Forestry, 69 (4): 91-112. doi:10.17707/AgricultForest.69.4.07

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AGRICULTURE AND ECONOMIC DEVELOPMENT OF THE AIT WERRA TRIBE DURING THE FRENCH COLONIALISM PERIOD AND ITS LOCAL CHARACTERISTICS (1912-1956) WITHIN THE MIDDLE ATLAS REGION OF MOROCCO

SUMMARY

The intertwining of agriculture and economic development during Morocco's period of French colonialism holds a fascinating historical significance. This paper attempts into how this relationship shaped the local characteristics of Morocco's economy, offering insights into the complex dynamics of colonial influence on agricultural practices and their impact on the nation's economic trajectory. This research aims to investigate the agriculture and economic development of the Ait Werra tribe during the French protectorate in Morocco. The French protectorate, established on March 30, 1912, brought about various changes in the agricultural and economic policies of Morocco in the first half of the twentieth century. These changes affected both urban and rural areas, but the tribal economy experienced the most significant impact. While the cities had already opened up to European countries and welcomed their traders, the Moroccan villages remained isolated from the outside world, and the authority of the Makhzen was not prevalent in many mountain tribes. Thus, colonization was a massive shock that transformed all aspects of life, especially for tribes like Ait Werra, which is a part of the Berber confederation and situated in the Middle Atlas Mountain range near to El Kssiba. This article will explore the fixations and transformations of the Ait Werra tribe's economy as it transitioned from the pre-Makhzen to post-colonial era in Morocco.

Keywords: agriculture; economic development; Ait Werra tribe; Atlas region; Morocco

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INTRODUCTION

Morocco, officially known as the Kingdom of Morocco, is situated in the north-western part of Africa within the historically significant Maghreb region. Spain is north of Morocco across the Gibraltar, and Algeria is on Morocco's eastern border (Petrovic and Reed, 2023; Muller *et al*, 2022). The history of Morocco during the French colonialism period is marked by a complex interplay of agricultural transformations, economic development, and the emergence of distinct local characteristics (Simonnet, 2007). This paper emphasis into the multifaceted relationship between agriculture, economic dynamics, and unique regional traits within the context of Morocco's colonial past (Pennell, 2000). By examining insights from scholarly works and historical records, this study aims to illuminate the ways in which French colonial policies influenced agricultural practices, shaped economic trajectories, and contributed to the nuanced social and economic fabric of local communities (Charis, 2012).

The Mediterranean region has been a pivotal cradle for the development of agriculture, showcasing a rich historical evolution from ancient times to the present day. The early agrarian practices of this region are notably highlighted by the emergence of the Neolithic period, around 10,000 BCE, witnessed through archaeological evidence such as the cultivation of cereals like wheat and barley, as well as the domestication of animals like sheep and goats (Zohary *et al.*, 2012; Luković, 2016; Gogić, 2023). This region's distinctive climate and geography played a significant role in shaping its agricultural trajectory, with the coalescence of innovative techniques like terracing and irrigation to counteract the region's aridity (Tainter, 2013). The Mediterranean's agrarian history is also closely linked to the diffusion of crops and ideas across civilizations, facilitated by the maritime networks that fostered exchange between ancient cultures like the Phoenicians, Greeks, and Romans (Bowman, 2000). Contemporary agriculture in the Mediterranean region is marked by a blend of traditional methods and modern technologies, adapting to challenges such as water scarcity and climate change while preserving its deep-rooted agricultural heritage (Food and Agriculture Organization, 2019). This enduring narrative of agricultural development in the Mediterranean underscores its pivotal role in shaping human civilization.

Agricultural policies under colonial rule had a profound impact on Morocco's economic landscape, shaping production systems, land distribution, and trade patterns. This influence extended to various regions and was often tailored to accommodate both French interests and local dynamics (Mundy, 2015). The tensions and negotiations surrounding these policies played a role in shaping the socio-economic dynamics of the time (Burke, 2017). This research aims to examine the economic transformations of the Ait Werra tribe during the French protectorate period (1912-1956) and highlighting the actual development of agriculture and economic policies. The case of the Ait Ouira tribe provides an illustrative example of how the French colonial agenda transformed the tribe's spaces and built environment (Mhenna, 1991). However, in much regions of Morocco and particularly in the Middle Atlas, each tribe had to be conquered for as general Guillaume said no tribe ever came in to French without its first having been beaten by force of arms. Lyautey, the first French Resident-General in Morocco from 1912 to 1925, aimed to conquer and make peace. One of them Bassou, was ruling the Ait Werra thirty years later with an influence extending far beyond his borders (Bidwell, 2012).

The objective of this study is to understand the impact of colonialism on local perceptions of Ait Werra's economic and cultural heritage. The imposition of French protection on Morocco on March 30, 1912, brought about significant economic developments during the first half of the twentieth century. France aimed to make Morocco an economic market and achieve this goal by controlling its economic mechanisms and modernizing its structures to meet the requirements of the capitalist regime. The economic development affected both Moroccan cities and villages, but the tribal economy experienced the most significant impact (El Bachir Bousslame, 1991). Unlike the cities, which had already opened up to European countries and welcomed their merchants, Moroccan villages remained isolated from the outside world, including the authority of the Makhzen, which was not prevalent in many mountain tribes (Gershovich, 2012). Therefore, colonialism was a shock that transformed all aspects of life, particularly for tribes like Ait Werra, whose economy was heavily affected. The significant research gap in post colonisation era focusing on agricultural sustainability has implicated the scientific and commercial growth in the study area. This article aims to analyze the constants and variables of the Moroccan tribal economy as a whole, between the pre-Makhzen and post-colonial eras basing on agriculture and economic activities, by studying the case of the Ait Ouira tribe, which located in the Middle Atlas of Morocco.

MATERIAL AND METHODS

Presentation of study area

Morocco is a country of the Maghreb located on the extreme North-West of the African continent and only 14 kilometres away from Spain (Bilgili and Weyel, 2009) along the North Atlantic Ocean and the Mediterranean Sea. Position of this area is strategic with the location along Strait of Gibraltar; and it is the only African country to have both Atlantic and Mediterranean coastlines (Sabri *et al*, 2019). The northern region of Morocco features mountainous terrain, including the Rif Mountains and the Atlas Mountains, alongside expansive plateaus interspersed with fertile valleys and coastal plains. In contrast, the southern part of the country is characterized by vast flat deserts, predominantly composed of rocky or sandy surfaces. The highest point is Jebel Toubkal 4,165 m, lowest point: Sebkha Tah -59 m; with mean elevation of 909 m. The main natural resources are phosphates, iron ore, manganese, lead, zinc, and fish, salt. Agricultural land is about 67.5%, arable land, 17.5%; permanent crops, 2.9%; permanent pasture, 47.1%; forest, 11.5%; other: 21%. The most significant population density in Morocco is concentrated along the Atlantic and Mediterranean coasts, with numerous densely populated urban areas dispersed across the Atlas Mountains (Moukhliss *et al,* 2022; Elfanne *et al*., 2022; Sabri *et al*, 2022; Abubakar, 2016; Abubakar, 2008).

Demographic profile

Morocco is experiencing a demographic transition characterized by a slower population growth rate, attributed to increased life expectancy and reduced birth rates among women. Enhanced healthcare, improved nutrition, better hygiene, and wider vaccination coverage have contributed to the decline in infant, child, and maternal mortality rates. Nevertheless, disparities persist, particularly between urban and rural areas as well as among households with varying levels of wealth. The decrease in Morocco's child population is a consequence of several factors, notably the decline in its total fertility rate, which has dropped from 5 in the mid-1980s to 2.2 by 2010. This reduction can be attributed to the rise in female educational attainment, increased contraceptive usage, delayed marriage, and a growing preference for smaller family sizes. A substantial portion of Morocco's population, nearly 26%, comprises young adults aged 15-29, presenting a significant potential economic workforce if gainfully employed. Nevertheless, the current reality shows that many of these youths face unemployment due to a disparity in job creation, which has not kept pace with the expanding working-age population. Those who do find employment are often engaged in the informal sector, where job security and benefits are typically limited (Moukhliss *et al*, 2022; Elfanne *et al.*, 2022; Abubakar, 2016; Abubakar, 2008).

In the second part of the 20th century, Morocco emerged as one of the leading countries in terms of emigration, resulting in the establishment of extensive and geographically diverse migrant communities in Western Europe. The Moroccan government has actively promoted emigration since gaining independence in 1956, for two key reasons: to secure remittances that can be used to support national development initiatives and to provide an outlet for preventing potential unrest in regions, often populated by Berber communities, with a history of rebellious tendencies. While Moroccan labour migrants had initially focused on destinations such as Algeria and France, a significant shift occurred from the mid-1960s to the early 1970s when Moroccan "guest workers" dispersed extensively across north-western Europe. Both host societies and the Moroccan migrants themselves initially anticipated that this migration would be temporary. However, due to worsening economic conditions in Morocco linked to the 1973 oil crisis and the implementation of stricter European immigration policies, these temporary stays evolved into permanent settlement.

A subsequent wave of family migration unfolded during the 1970s and 1980s, marked by a rising number of second-generation Moroccans choosing to pursue naturalized citizenship in their host countries. In the mid-1980s, Spain and Italy emerged as new destinations for Moroccan migrants. However, the early 1990s saw both countries imposing visa restrictions, prompting Moroccans to increasingly migrate through legal means by marrying individuals of Moroccan descent already residing in Europe or resorting to illegal channels to engage in underground economy activities. Notably, there was a growing representation of women among these labour migrants. Simultaneously, some highly skilled Moroccans sought opportunities in the United States and Quebec, Canada.

During the mid-1990s, Morocco evolved into a transit hub for asylum seekers originating from Sub-Saharan Africa and undocumented labour migrants hailing from Sub-Saharan Africa and South Asia. They aimed to reach Europe, primarily by crossing into southern Spain, the Spanish Canary Islands, or Spain's North African enclaves, Ceuta and Melilla. Despite instances of forceful expulsions by Moroccan and Spanish security forces, these illegal migrants have persisted in their attempts, and concerns over security in Europe have not been assuaged. Rabat remains reluctant to endorse a European Union agreement to repatriate third-country nationals who have entered the EU illegally via Morocco. Meanwhile, many other undocumented migrants have opted to remain in Morocco either temporarily, while they accumulate sufficient funds for onward travel, or as a "second-best" permanent choice. The initiation of a regularization program in 2014 conferred legal status upon some migrants, granting them equal access to education, healthcare, and employment opportunities.

Age structure

Based on the reports available at the national statistics, but also to the publications of various authors (Moukhliss *et al*, 2022; Elfanne *et al*., 2022; Abubakar, 2016; Abubakar, 2008), population with the age of 0-14 years is 26.01% (male 4,919,266/female 4,722,463); 15-64 years: 65.92% (male 12,124,939/female 12,311,552); 65 years and over: 8.06% (2023 est.) (male 1,455,355/female 1,533,845).

Total dependency ratio is 52.2; youth dependency ratio: 40.9; elderly dependency ratio: 11.3; potential support ratio: 8.9. Median age total is 29.1 years; where male are 28.7 years, female is 29.6 years; and population growth rate is 0.88%; Birth rate, 17.1 births/1,000 population; 6.61 deaths/1,000 population.

Major urban areas are located with 3.893 million in Casablanca, 1.959 million RABAT (capital), 1.290 million Fes, 1.314 million, Tangier, 1.050 million Marrakech, and 979,000 Agadir. Sex ratio at birth is 1.05 male(s)/female, having the following structure for the age of 0-14 years: 1.04 male(s)/female; 15-64 years: 0.98 male(s)/female; 65 years and over: 0.95 male(s)/female; total population: 1 male(s)/female (2023 est.). Maternal mortality ratio is 72 deaths/100,000 live births; and total: 18.73 deaths/1,000 live births: male: 20.94 deaths/1,000 live births; female: 16.41 deaths/1,000 live births. Life expectancy at birth for the total population is 73.95 years with male: 72.26 years and female: 75.72. Total fertility rate is 2.27 children born/woman.

Natural hazards

In the northern region, the mountains exhibit geological instability, making them susceptible to earthquakes. This area also experiences periodic droughts, windstorms, flash floods, and landslides (Bammou *et al.*, 2023). The prolonged existence of natural hazards could inflict the soil quality in terms of loss in NPK (Kader *et al*, 2022a; Kader *et al*, 2022b), mechanical strength of soil, stormwater retention capacity (Kader *et al*, 2023a), and it necessitates the additional investments to investigate alternate materials such as animal digestates in terms of their viability to become sustainable fertilisers for agricultural crops (Chozhavendhan *et al.*, 2023; Kader *et al*, 2022). In the southern part, the winter and spring seasons may bring hot, dry sirocco winds laden with dust and sand. Furthermore, approximately 60% of the time, a persistent harmattan haze can severely limit visibility, creating challenging atmospheric conditions (Ouallali *et al.*, 2020).

The Aït Ouirra tribe

The Aït Ouirra, a Berber tribe, occupy an area at the heart of Morocco that is approximately 17 km in width and 35 km in depth, stretching from the crests of the northern slopes of the Oued El Abid to the Oum Err Bia rivers (Ennaji *et al*, 2022). The total area of this territory is about 600 km^2. The tribe is belonging to Beni Mellal Khenifera region.

The Aït Ouirra tribe is a significant and representative faction of the Aït Seri bled, situated at the centre of the Middle Atlas in Morocco, equidistant from major cities (Vaugien, 1951). The region's specific traits, such as its climate and location, historically made it ideal for livestock operations, and double transhumance was a traditional practice. However, the French intervention during the colonial period had a strong impact on this phenomenon. On the other hand, Ait Ouira tribe inhabit a zone that serves as a contact and transition point between Arab and Berber regions, as they reside in a Berber area while also being influenced by the neighbouring Arab plain (Figure.1). Throughout its history, the significance of this tribe has been reinforced by its geographic location, and our aim is to document its development in order to gain a comprehensive understanding of its evolution. The tribe was based on agriculture especially grazing, exploiting the large forest area of the Middle Atlas (Ouakhir *et al.*, 2020). According to the legend reported by Berber scholars of the Great Atlas, the Aït Ouirra originated from a poorly defined country neighbouring Tafilalet (A.D.N 1926 et 1953), Figure 1.

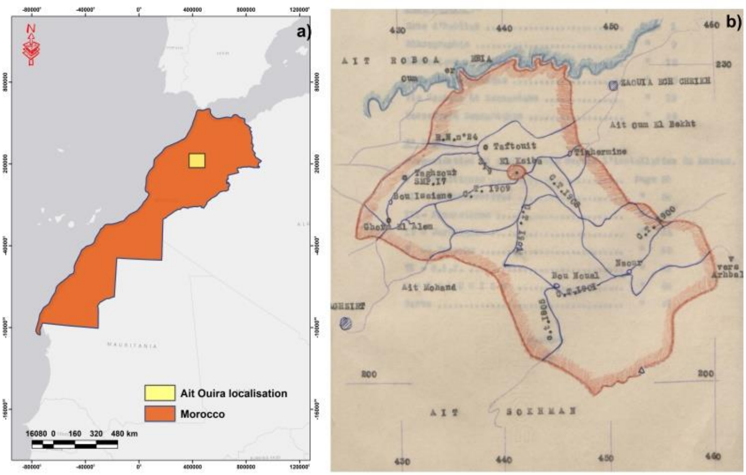

Figure 1: The location of Ait Werra in the Middle Atlas of Morocco and the study area of the Ait Werra tribe (A.D.N, 1953)

Methodology and data

The adopted methodology in this article employs multiple approaches to address the research issue and analyse the obtained data from the French Archives in Nantes. The first approach is the historical method, which involves three crucial elements for studying any historical subject: time, space, and event. The second approach relates to the French Annals School, whose pioneers, led by Fernand Braudel, advocate asking contemporary questions on old issues, as the present and past can shed light on each other. Additionally, the third method is the economic approach, which combines history and economics. This approach was necessary to digitize and analyse data in a manner that suits the economic understanding of the period being studied. Furthermore, the fourth approach focuses on the anthropological method, which seeks to understand a society that largely preserved many primitive customs and traditions.

In order to comprehensively understand the subject, various historical archives were consulted at both national and international scales. The most notable archives used in this research include:

–The unclassified archive of the tribe, containing historical documents from the French protectorate period in Morocco. This archive was studied for a period of three months and is stored in the court, municipality, and community of El Kssiba (Morocco).

–The French National Diplomatic Archive in Nantes, which houses documents from the French colonies, including a dedicated section for Moroccan tribes. I visited this archive in January and February of 2021 and used the source material obtained from it to complete and finalize this article (Table.1).

Table 1: Presentation of the used archive and data in this study (1912-1956)

Archive name	Date	Source of archive
French diplomatic archive in Nantes (A.D.N)	1934- 1953	https://www.diplomatie.gouv.fr/
Archive of El Kssiba Municipality	1924-1656	El Kssiba (Morocco)
Archive of El Kssiba Tribunal	1929-1956	El Kssiba (Morocco)

RESULTS AND DISCUSSION

Climatic context of the studied area

The climate of Morocco is Mediterranean in the north, becoming more extreme in the interior; in the south, hot, dry desert; rain is rare; cold offshore air currents produce fog and heavy dew (Moukhliss *et al*, 2022; Elfanne *et al.*, 2022). The climate of El Kssiba is temperate and rainy, with some years receiving more than 1000 mm of rainfall. This weather is ideal for growing fruit trees, such as olive and orange trees, which thrive in these conditions (Bouet 1938). In addition, the water that flows down the mountain slopes enables high-yield irrigated crops like wheat, barley, corn, and pepper, as well as supporting a thriving livestock industry (Figure.2).

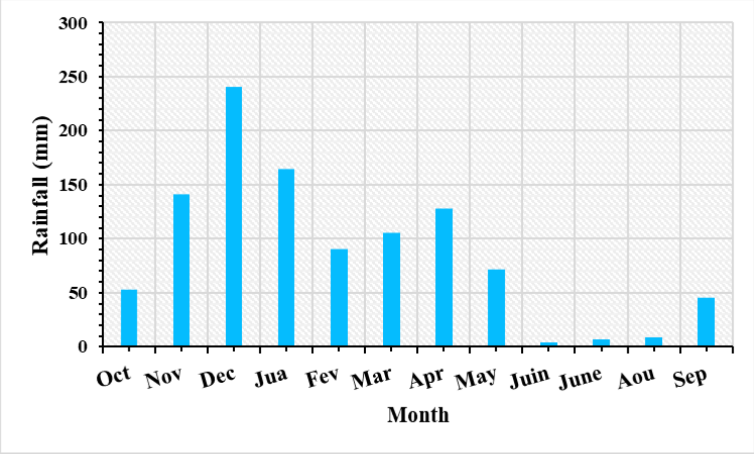

Figure 2: Monthly rainfall during the studied period (1942- 1952)

Source: Ibid 1948

The piedmont (Dir) is a part of the Oum Er Bia basin, which receives water from the northern slopes of the Middle Atlas, particularly during the rainy season, including the Chef n Goub, the oued Bou Zabel, and the oued Zemkil (Ouakhir and El Ghachi, 2023). However, these streams cannot be replenished by the springs whose water is used entirely for irrigation, despite the abundance of water (Mengaud 1932; Kader *et al*., 2023), illustrated by Figure 3.

Figure 3: Water resources situation in Ait Werra tribe, 1952 (*A.D.N, 1953*)

Ait Werra tribe between agriculture and grazing activities

Agriculture

The Ait Werra tribe is known for its agricultural estates that cultivate various types of utility trees, such as olive, orange, cherry, and almond trees. Although these crops are mainly for subsistence purposes, they are also sold in local markets in the Ait Werra, Takezirt, and Fashtala areas since the 9th century

(R.M.E.P 1920, 1924 et 1926), Figure 4. Additionally, the area has irrigated agriculture, with some types of cereals still grown for local consumption. The reason for the locals' attachment to agriculture despite Ait Werra's primarily pastoral nature is the abundance of water resources from the surrounding mountains (Archive of El Kssiba Municipality 1933).

A SWOT analysis for the agriculture in the Ait Werra tribe can be summarized as follows:

Strengths:

Abundance of Water Resources: The region's access to abundant water resources from the surrounding mountains is a significant strength. This enables irrigation and sustains agricultural activities even in areas where agriculture might not be traditionally viable.

Historical Agricultural Tradition: A long history of agricultural cultivation, dating back to the 9th century, signifies a deep-rooted tradition of farming in the Ait Werra tribe. This tradition provides valuable knowledge and experience in crop cultivation.

Variety of Utility Trees: The cultivation of various types of utility trees, including olive, orange, cherry, and almond trees, diversifies the agricultural production and potentially provides multiple sources of income.

Weaknesses:

Subsistence Focus: While agriculture is a part of the local economy, the primary focus is on subsistence farming. This may limit the potential for income generation and economic growth from agricultural activities.

Limited Market Access: Selling crops mainly in local markets may limit revenue opportunities. Expanding access to broader markets could potentially increase profits.

Opportunities:

Diversification of Income: The cultivation of a variety of utility trees offers an opportunity to diversify income sources, especially if there is a focus on enhancing production and marketing strategies.

Value Addition: Processing and adding value to agricultural products, such as producing olive oil or preserves from fruits, can create higher-value products for sale, increasing revenue.

Agricultural Education and Modernization: Investing in agricultural education and modern farming techniques could lead to increased productivity and income potential.

Threats:

Climate Change and Environmental Risks: Climate change and related environmental risks, such as droughts or pests, can have a significant impact on crop yields and agricultural sustainability.

Market Fluctuations: Dependency on local markets exposes the agricultural sector to fluctuations in demand and prices, making income unstable.

Competition: Increased competition from other regions or agricultural producers could threaten the viability of local agriculture, especially if they have access to more advanced farming practices and markets.

The agriculture in the Ait Werra tribe has strengths in terms of water resources, historical tradition, and crop diversity. However, there are weaknesses related to subsistence focus regarding the influential parameters to enhance the soil quality and limited market access. Opportunities exist in income diversification, value addition, and modernization, but the sector faces threats from climate change, market fluctuations, and competition. Successful development of agriculture in the region will depend on efforts to mitigate weaknesses and capitalize on opportunities while addressing potential threats.

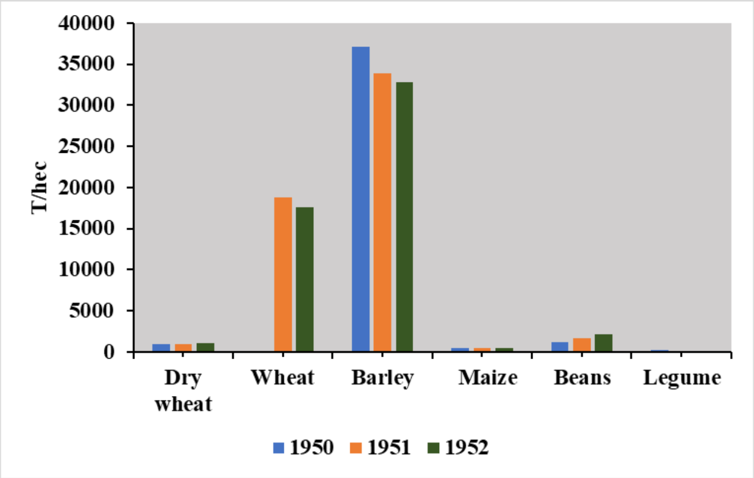

Figure 4: The amount distribution of the cultivated species during the period of 1950, 1952 and 1952. (*A.D.N*, *1953*)

Livestock

Caid Moha Ou Said played a crucial role in securing the extraction of Ait Werra and Umm Al-Bakhth by negotiating agreements with the tribes of the Tadla plains and Beni Mellal prior to the French protection of Morocco in 1912. However, after the imposition of colonial control over the region, pro-colonial rulers exploited pastoral lands by preventing nomads from accessing them (A.D.N 1926), Figure 5.

Figure 5: Overgrazing and traditional labour in the Middle Atlas of Morocco (Photo: Amraoui)

This caused the nomads of Ait Werra and neighbouring tribes to seek permission from landowners and influential figures to access the areas they previously benefited from under comprehensive agreements for all tribes of the Dir and Mountainous areas. The colonial administration aimed to maintain the nomadic movement and win them over through civil policies. Additionally, the administration released the hands of influential figures to expand their possessions in exchange for their support in eliminating rebellious tribes and penetrating the mountains. The subjugated factions were also permitted to rebuild their herds of livestock after opening markets in the Tadla region (Guennoun 2014). Due to the fact that Ait Werra does not produce fodder and instead relies on grazing, their livestock is highly susceptible to climatic conditions, particularly rainfall. The recent drought has had a significant impact on the tribe, leading to a drastic decrease in the number of livestock from 13,000 in 1942 to only 6,355 in 1952 AD. Additionally, horse breeding is limited in this area, and mules and donkeys are often used as a replacement (Eyraud 1934) (Figure.6).

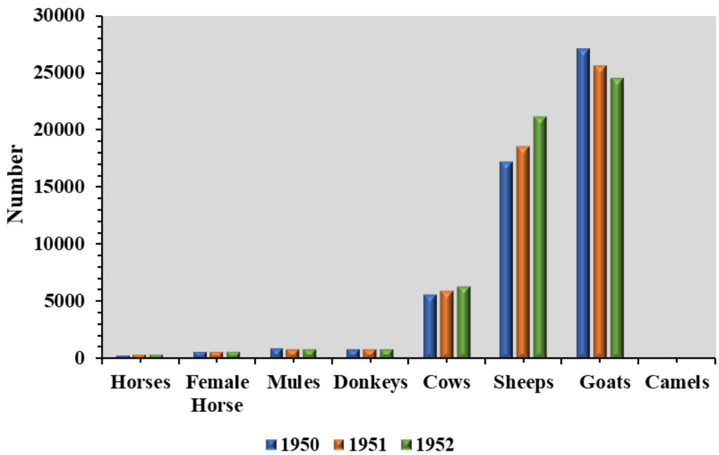

Figure 6: Distribution of the number of livestock during the studied period (A.D.N, 1953)

Figure 7: Place for feeding livestock in 1952 and 2022

A SWOT analysis for the livestock situation in the Ait Werra tribe can be summarized as follows:

STRENGTHS:

Historical Agreements: Historical agreements secured by figures like Caid Moha Ou Said allowed the Ait Werra tribe access to pastoral lands, providing a foundation for their livestock-related activities.

Colonial Policies: The colonial administration's policies aimed at maintaining nomadic movement and supporting livestock herders by allowing them to rebuild their herds after opening markets in the Tadla region contributed to the recovery of livestock numbers.

Traditional Knowledge: The Ait Werra tribe has traditional knowledge and experience in livestock management and pastoral practices.

WEAKNESSES:

Dependency on Rainfall: The tribe's reliance on grazing, rather than fodder production, makes their livestock highly susceptible to climatic conditions, particularly rainfall. Droughts, like the recent one mentioned, can have a severe negative impact on livestock numbers.

Limited Horse Breeding: Limited horse breeding in the area can be a weakness, especially if horses are needed for specific agricultural or transportation purposes.

OPPORTUNITIES:

Diversification of Livestock: Exploring the possibility of diversifying the types of livestock rose, such as exploring alternative breeds that are more resilient to local conditions, could be an opportunity for the tribe.

Fodder Production: Developing methods for fodder production, even in arid conditions, could help mitigate the vulnerability of livestock to climatic fluctuations.

Market Access: Leveraging market access in the Tadla region can present opportunities for trade and income generation through livestock sales.

THREATS:

Climate Change and Drought: Climate change-related challenges, including increased drought frequency and severity, pose a significant threat to the tribe's livestock herds.

Limited Access to Resources: Ongoing challenges related to access to pastoral lands, especially if influenced by external factors or policies, could threaten the tribe's ability to sustain its livestock practices.

Competition: Competition from other livestock producers, especially those with more diversified herds or access to better resources, may pose a threat to the tribe's livestock industry.

The livestock situation in the Ait Werra tribe has historical strengths and policy support that have helped sustain their livestock herds. However, weaknesses include vulnerability to climatic conditions and limited horse breeding. Opportunities lie in diversifying livestock and improving fodder production, while threats include climate change, resource access issues, and competition from other producers. Adaptation and resilience-building strategies are essential to address these challenges and ensure the sustainability of livestock-related activities in the region.

Crafts and traditional industry

Handicrafts in the region of Ait Werra, located in El Kessiba, are concentrated in the urban areas which have a high population of 2045 inhabitants. The tribe is well-known for its diverse range of crafts, with some of the most prominent ones being tanning, manufacturing of soles and leather bags, and pottery (R.M.E.P 1924).

(i) Tanning, which involves providing ready-made leather, has been a major source of prosperity for the tribe, particularly during the Second World War. However, this craft has faced a significant decline in recent times. It mainly supplies leather to Naali, a maker of Al-Balagha babouchiers in El Kessiba and Beni Mellal, as well as to leather bag makers.

(ii) The manufacturing of soles and leather bags involves thirteen makers and eight apprentices in El Kssiba. They produce around 4,000 soles and 1,600 leather bags annually. However, this craft faces high production costs due to the use of materials brought from outside the tribe. Additionally, the local demand for their products is low, except for women's slippers which are mostly sold in the local market.

(iii) Potters in the region are also abundant, with ten located in El Kessiba, two in Taftiout, two in Aghram Al-Alam, and one in Al-Jabal. The craft has flourished greatly due to the abundance of raw materials available. They produce an estimated 3600 cooking cookers (service), 1500 couscous, 3000 Farah (a large bowl for bread), 6000 milkmaids (a bowl of water), 1800 charcoal heaters per month (majmar), and 5,000 small stoves for heating annually (A.D.N 1953).

Trade and Markets

In August 1924, immediately after tightening control over the area of Ait Werra, the French authorities took the initiative to organize a weekly market in Al-Kosiba on Sundays. This was the first time such an event had been held since the French occupation of the region. Military reports suggest that this step was successful, and it attracted many residents from the surrounding mountain areas (Marché Lainier d'El Kessiba 1939).

The population responded positively to the organization of the weekly market, as it provided them with the opportunity to sell their goods and purchase commodities and agricultural products. The French authorities allowed residents to use their plots of land for the market facilities. In 1936, the people of El Kssiba donated a land area of 3465 square meters to expand the weekly market.

The market is divided into several sections, each dedicated to selling specific products, and each section is called *Al-Rahba*. These sections are as follows:

-The Wool Market showcases various types of wool for sale, with a total of about thirty tons sold in 1939 AD, mostly by the sheep, goat, and cow breeding cooperative that holds a monopoly. The wool is sold by the "*Telest" unit.*

-The Grain market is dedicated to selling wheat, barley, maize, and beans, and is usually located on the side of the market. The selling unit is known as the Abra.

-Livestock market is a place where traders gather to sell cows, sheep, and goats. It is located adjacent to the market's slaughterhouse and the place where families grill meat during their visit to the market.

-The markets in the Tadla region are organized on different days of the week in various locations. Kasbah Tadla, Foum El-Anr, and Foum El-Juma'a are held on Mondays. Tanougha and Wauzegat are held on Tuesdays. Aghbala, Zawiya of Sheikh Weinerki, and Iqbali and Atab are held on Wednesdays. Tagezirt, Azilal, and Bezo are held on Thursdays. Tadla, Beni Mellal, Beni El Ouidane, and Ait M'hamed are held on Fridays. Lastly, Welad Said El-Wad is held on Saturdays, while El Kessiba, Ben El Ouidane, Takleft, Afourar, and Zawiyat Ahansal are held on Sundays (Bulletin de la Chambre d'agriculture de Casablanca 1950).

A study in attempts to modernize the economy of Ait Werra

Associations and cooperatives

The French authorities have encouraged the formation of agricultural associations and cooperatives in the Ait Werra tribe, which represents a significant change in the region. Prior to this, the region had not experienced the creation of such associations or cooperatives. Below are some examples of such cooperatives and associations (Vaugien, 2014):

-An agricultural production cooperative was created in 1937 in the presence of the chief of the Ait Werra community. Its president was a person from the Ait Hadidou tribe who was committed, according to the minutes of the cooperative, to provide all the necessary necessities to the owners of the land belonging to the cooperative, such as seeds, machinery, and medicines.

-The Water Mill Association was registered in 1939 in partnership between Maymoon, Zain, and Yamna Ahadah. The association's internal regulations defined all production operations from the mill stage to the production stage, followed by the profit division stage.

-There were two livestock breeding cooperatives that had been established. The first one was founded on June 4, 1951, and its members were two people from Ait Werra: Saeed Umimon, Khalaf, and Ali. The first provided the second with thirty heads of sheep for their care. After two years, the two parties would sell their products and share the profits after deducting the expenses. The second Livestock Breeding Cooperative was registered on August 13, 1951, at the Martial Court of Ait Werra, between an investor from Kasbah Tadla and a sheriff from Ait Werra. The first party bought six cows from the second for 20,500 francs to be cared for over a year, with a fifty-fifty share in the profits (Tribunal coutumier des Ait Werra 1951). A SWOT analysis for the study in attempts to modernize the economy of Ait Werra through the formation of associations and cooperatives can be summarized as follows:

STRENGTHS:	WEAKNESSES:
Economic Modernization: A positive step towards modernizing the economy by introducing new practices, and potentially improving stability. ***Resource Provision:*** The agricultural production cooperative's commitment to providing necessities like seeds, machinery, and medicines to landowners is strength, as it supports local farmers and enhances agricultural productivity. ***Collaborative Efforts:*** The formation of the Water Mill Association and livestock breeding cooperatives highlights collaborative efforts, which lead to resource & knowledge exchange.	***Limited Historical Experience:*** The region had no prior experience with such associations or cooperatives, which could lead to challenges in terms of knowledge and capacity for effective management and operation. ***Profit Division Complexity:*** The complex profit division process within the Water Mill Association and the livestock breeding cooperatives may lead to disputes or misunderstandings among members, potentially undermining their effectiveness.
OPPORTUNITIES:	**THREATS:**
Economic Diversification: The creation of these associations and cooperatives presents an opportunity for diversifying the local economy beyond traditional agricultural practices, potentially leading to increased income and economic resilience. ***Capacity Building:*** With time and experience, members of these associations and cooperatives can develop skills and knowledge in modern agricultural and business practices, enhancing their ability to manage and operate such entities effectively.	***Resistance to Change:*** May face resistance from traditionalists or those who are sceptical of such changes, which could hinder their success. ***Lack of Legal Framework:*** May not have been well-defined, which could pose legal and regulatory risks. ***Market and Environmental Risks:*** Market fluctuations and environmental challenges can pose threats to the success of these cooperatives.

The efforts to modernize the economy of Ait Werra through the formation of associations and cooperatives have the potential to bring about economic benefits and collaborative opportunities. However, they also face challenges related to limited historical experience, profit division complexity, resistance to change, legal issues, and external market and environmental risks. Successful implementation and long-term sustainability will depend on effective management, capacity building, and adaptation to changing conditions.

Agriculture Directorate (Agricultural Modernization Sector)

France established the Directorate of Agriculture in the Ait Werra tribe with the aim of controlling the agricultural sector in the region and making it a tool to bring the people closer to the colonial administration (Tribunal coutumier des Ait Werra 1954).

The local authorities submitted a request to establish it in 1946, and the responsibility for its construction in Taghazout in August 1946 was assumed by its first director, Vincent, who officially completed it on 16 April, 1947 (Tribunal coutumier des Ait Werra (1937).

It had several appendices in the field of El Kssiba Department, namely:

- In the thigh of Ait Hussein on the communal land N° 17.027/c
- In the thigh of Ait Hussein or Yacoub on communal land N° 23.120/c

- In the thigh of Ait Hami on the communal land N° 17.025/c
Extensions in Aghbala and Zawiyat E-Sheikh (A.D.N 1953).

Colonial private companies

Colonial company

The National Reserve Company was established in El Kssiba on 25 July 1927 AD as an alternative to the company that was dissolved in Abi Al-Jaad in the same year. This trend is evident in the number of times in which the composition of this company was amended, especially during 1930 and 1936 (Arrêté ministériel 1937).

The company continued to play large economic and social roles, and its value increased until it became eight branches with its headquarters in El Kssiba, Ait Sakhman Oriental branch, and Ait Hadidou branch. In the year 1941 AD, El Kssiba Company was reduced to six branches by deleting the branches of Ait Dawood o Ali and Ait Hadidou (Arrêté ministériel 1941).

El Kssiba metal company

This company was established in El Kessiba in the forties of the last century after the discovery of large quantities of lead metal in the Ait Werra Mountains, where the colonial authorities began paving the roads leading to this mineral, and the people called this area Bo Al-Maadin. The colonial administration exploited this mineral by transporting it to the coasts as a raw material and exporting it to France. The Ouiraouian Labor force was also exploited at very low prices in what is known as rock work (Arrêté ministériel 1955). After that, the company expanded its activities, as it submitted a request to the French authorities in December 1954 to grant it the right to benefit from the benefits of licenses granted to various investors in the mineral field (The oral narration, 2021).

Table 2: Distribution of Some reforms were done by colonial authorities

The budget cover (*Franc*)	Reforms
50000	Water fountain
75000	Repair of the water system
850000	Additional shower and toilet
20000	Repair of a bridge on the valley of Taghbalot
300000	Building 2 showers near the toilets of Taghbalot
55000	Repair of clothing warehouses for bathrooms and swimming pools
330000	Repairing some wooden houses
20000	Transformations of the water direction of Tagbalut sources
50000	Building a water sink
180000	Painting some wooden houses and restoring them

Encouraging mountain tourism in the area of Ait Werra

The colonial authorities attended to the tourism aspect of El Kessiba and its surroundings by:

-Establishing the Tagbalout Resort in the 1940s, positioned between Tizi Night Ouerra and El Kessiba, and outfitting it with hotels and wooden rental houses.

-Releasing a decree on December 24, 1939, which mandated the acquisition of written authorization from regional authorities for reserving accommodations at the El Kessiba Hotel or a wooden dwelling in Tagbalout?

-This measure aimed to bolster protective measures for the frontier in the Ait Werra Mountains, prompted by the security deterioration experienced by the region towards the late 1930s. It was applied even to French families who travelled to Kessiba for weekends from nearby areas like Tadla and Beni Mellal.

-In 1943, El-Kessiba received funding for summer camps and resorts, with the allocated credit amounting to 500,000 francs.

-In view of the success achieved by Taghbalut Resort and the increase in its annual income, the colonial authorities decided in 1955 AD to rehabilitate it and allocated the following budget for it.

-A SWOT analysis for the colonial authorities' actions related to tourism in El Kessiba and its surroundings can be broken down as follows:

STRENGTHS:

Strategic Location: El Kessiba and its surroundings were strategically located, making it an attractive destination for colonial authorities to invest in tourism development.

Establishment of Tagbalout Resort: The creation of the Tagbalout Resort in the 1940s with hotels and wooden rental houses showcased a commitment to developing tourism infrastructure in the region.

Security Measures: The decree mandating written authorization for accommodations demonstrated a commitment to security and control in the area, which was important during a period of security deterioration.

Allocation of Funding: The allocation of 500,000 francs in 1943 for summer camps and resorts in El-Kessiba indicated financial support for the development of tourism facilities.

WEAKNESSES:

Colonial Exploitation: The colonial authorities' actions may be seen as exploiting the region for their own benefit, primarily as a means to secure the frontier and maintain control rather than for the well-being of the local population.

Limited Local Engagement: There is no mention of involvement or consultation with the local population in the development of tourism infrastructure, which could lead to potential conflicts or resentment.

OPPORTUNITIES:

Economic Growth: The success of the Tagbalout Resort and the increase in annual income suggests the potential for economic growth through tourism in the region.

Rehabilitation of Tagbalout Resort: The decision to rehabilitate the resort in 1955 indicates ongoing commitment to tourism development, potentially attracting more visitors and revenue.

THREATS:

Environmental Concerns: There is no mention of environmental considerations in the development of tourism infrastructure, which could lead to long-term ecological issues if not addressed.

The colonial authorities' actions in developing tourism in El Kessiba and its surroundings had strengths in terms of strategic location and initial investment. However, they also had weaknesses in terms of potential exploitation and limited local engagement. The opportunities included economic growth and the rehabilitation of existing facilities, while threats encompassed political and social unrest, changing politics, and environmental concerns.

CONCLUSION

In conclusion, this article unveils the intricate interplay between agriculture, economic development, and local distinctiveness within the context of Morocco's colonial history under French rule. Through a focused exploration of the Ait Werra tribe in the Middle Atlas Mountains, the study elucidates the profound influence of French colonial policies on agricultural practices and economic trajectories. The establishment of the French protectorate in 1912 ushered in substantial changes to Morocco's economic and agricultural landscapes, particularly impacting tribal economies. While urban centres absorbed European influences, the villages, notably those belonging to mountain tribes like Ait Werra, grappled with transformative disruptions resulting from colonization.

By dissecting the evolution of Ait Werra's economy from the pre-Makhzen era to the post-colonial period, encompassing elements such as agriculture, livestock, crafts, and trade, this research effectively employs historical records, archives, and rigorous economic analysis. As a result, it sheds crucial light on how colonial interventions intricately moulded not only the economic fabric but also the cultural heritage of Ait Werra and akin communities during this pivotal transformative phase. In sum, the article underscores how the historical synergy between colonial policies and local responses indelibly shaped the trajectory of Ait Werra's economy and culture. This nuanced exploration serves as a testament to the enduring legacy of colonialism, illustrating how its echoes continue to reverberate through economic structures and cultural identity in contemporary times. These developments impacted both Moroccan cities and villages, although the tribal economy felt the most significant impact. Unlike the cities, which had already embraced European traders and experienced an opening towards European countries, Moroccan villages remained isolated from the outside world, including the authority of the warehouse, which was not widespread in many mountain tribes. Colonialism, therefore, was a shock to them that altered all aspects of their lives.

This article examines the constant and variable elements of the Moroccan tribal economy as a whole between the past Makhzen and the present colonial era, focusing on the Ait Werra tribe of the Middle Atlas.

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REFERENCES

A.D.N (1926). 1MA/285/57- 35.3, « Étude sur les Ait Werra et Ait Abdi » Bureau des renseignements, Lieutenant Schweitzer chef du Bureau des renseignements – El Kssiba. P 13.

A.D.N (1953). article 1MA/285/2-67.67bis « Fiche de la tribu des Ait Werra. A.D.N 1953 » Affaires indigènes, les capitaine Boss et Valache, chefs de bataillon du Cercle d'El Kssiba, N° 391, 20 février. P 2.

Abubakar, B. (2008). The Perception of Archaeology in Africa: Opportunities and Potential, IJNA 37.2, 387-388.

Abubakar, B. (2016). Sahara Desert Treasures, Geography and Associated Wonders: Yesterday, Today and Tomorrow.

Archive of El Kssiba Municipality (1933). Correspondance De capitaine d'ales commandant du cercle d'el kassiba au colonel commandant le cercle d'El Kassiba. N 90/OP. 15 Aout.

Arrêté ministériel (1937). Le Journal officiel numéro 1293, 6 août 1937, p. 1335.

Arrêté ministériel (1941). Le Journal officiel, numéro 1484, 4 avril, p. 563.

Arrêté ministériel (1955). Journal officiel n° 2216, 15 avril 1955, p. 1032.

Bammou Y., Bouskri I., Brahim B., Kader S., Brahim I., Abdelkrim B., Spalevic V. (2023). The contribution of the frequency ratio model and the prediction rate for the analysis of landslide risk in the Tizi N'tichka area on the national road (RN9) linking Marrakech and Ouarzazate. Catena, 232, 107464. doi: 10.1016/j.catena.2023.107464.

Bidwell, R. (2012). Morocco under colonial rule: French administration of tribal areas 1912-1956. Routledge.

Bilgili, Ö., & Weyel, S. (2009). Migration in Morocco: History, Current Trends and Future Prospects. Paper Series: Migration and Development Country Profiles, December 2009, Maastricht Graduate School of Governance, 1-62.

Bouet, G. (1938). Nouvelles recherches sur les Cigognes blanches de l'Afrique du Nord. L'oiseau et la Revue française d'ornithologie. VOL. VIII. — N° 1. Imprimerie centrale (louis laboureur) P 24.

Bowman, A. K. (2000). The archaeology of Mediterranean prehistory. Blackwell Publishers.

Bulletin de la Chambre d'agriculture de Casablanca (1950). État des souks de la région de Casablanca, (publicité). Vingt-Et-Unieme Annee. N° 233, Septembre, P 39.

Burke, E. (2017). Prelude to Protectorate in Morocco: Precolonial Protest and Resistance, 1860-1912. Routledge.

Charis, M. (2012). French Colonial Agricultural Policy and the Legend of "Colonial Development". Agricultural History, 86(1), 102-124.

Chozhavendhan, S., Karthigadevi, G., Bharathiraja, B., Praveen Kumar, R., Abo, L. D., Venkatesa Prabhu, S., Ramalingam, B., Jayakumar, M. (2023). Current and prognostic overview on the strategic exploitation of anaerobic digestion and digestate: A review. Environmental research, 216, 114526. doi:https://doi.org/10.1016/j.envres.2022.114526

El Bachir Bousslame, M. (1991). Histoire de la tribu de Béni Mellal (1854-1916). New Knowledge Press, Rabat 1991 AD.

Elfanne, O.K., Boutchkkoucht, A., El Hassani, F., Elmoufid, R., El Fathi, B., Spalevic, V., Ouallali, A., Benhachmi, M.K. (2022). Climate change and decision support using the computer tool InStat for El Ganzra Region, Morocco. Agriculture and Forestry, 68 (3), 199-220. doi:10.17707/AgricultForest.68.3.16.

Ennaji, N., Ouakhir, H., Halouan, S., & Abahrour, M. (2022). Assessment of soil erosion rate using the EPM model: case of Ouaoumana basin, Middle Atlas, Morocco. IOP Conference Series: Earth and Environmental Science, 1090(1). https://doi.org/10.1088/1755-1315/1090/1/012004

Food and Agriculture Organization. (2019). Regional Overview of Food Security and Nutrition in Europe and Central Asia 2019: Accelerating Progress Towards the SDGs. FAO.

Gershovich, M. (2012). French military rule in Morocco: Colonialism and its consequences. Routledge.

Gogić, M. (2023). An overview of livestock breeding in the medieval Zeta through the legal norms of the statutes of Kotor, Budva, and Skadar. Agriculture and Forestry, 69 (2), 203-215. doi:10.17707/AgricultForest.69.2.16.

Guennoun, S. (2014). La Montagne Berbère: les Aït Oumalou et le pays Zaïan. Rabat: Omnia, P 17.

Kader, S., Chadalavada, S., Jaufer, L., Spalevic, V., & Dudic, B. (2022). Green roof substrates-A Literature review. Frontiers in Built Environment, 8. doi:https://doi.org/10.3389/fbuil.2022.1019362

Kader, S., Jaufer, L., Bashir, O., & Raimi, M. O. (2023). A Comparative Study on the Stormwater Retention of Organic Waste Substrates Biochar, Sawdust, and Wood Bark Recovered from Psidium Guajava L. Species. Agriculture and Forestry, 69(01), 105-112. doi:https://doi.org/10.17707/AgricultForest.69.1.09

Kader, S., Novicevic, R., & Jaufer, L. (2022). Soil Management In Sustainable Agriculture: Analytical Approach For The Ammonia Removal From The Diary Manure. Agriculture & Forestry/Poljoprivreda i Sumarstvo, 68(4), 69-78. doi:10.17707/AgricultForest.68.4.06

Kader, S., Raimi, M. O., Spalevic, V., Iyingiala, A.-A., Bukola, R. W., Jaufer, L., & Butt, T. E. (2023). A concise study on essential parameters for the sustainability of Lagoon waters in terms of scientific literature. Turkish Journal of Agriculture and Forestry, 47(3), 288-307. doi:10.55730/1300-011X.3087

Kader, S., Spalevic, V., & Dudic, B. (2022). Feasibility study for estimating optimal substrate parameters for sustainable green roof in Sri Lanka. Environment, Development and Sustainability, 1-27. doi:https://doi.org/10.1007/s10668-022-02837-y

Luković, M. (2016). Self-Government Institutions of Nomadic and Semi-Nomadic Livestock Breeders in the Balkans and in the Carpathian Regions in the Late Medieval and Early Modern Periods. Res Historica, 41, 51-94.

Marché Lainier d'El Kessiba (1939). Le Journal Le Courrier colonial. N° 2057, 6 juillet, P 2.

Mengaud, L. (1932). « Géologie - sur la structure de la chaîne cantabrique ». Comptes rendus hebdomadaires des séances de l'acadëmie des sciences, tome 180. Gauthier-Villars Imprimerie Librairie, Paris, P 1095.

Mhenna, H. (1991). Systèmes de production et de gestion de l'espace chez les Ait Werra (Moyen Atlas). Mémoire de 3éme cycle pour l'obtention du diplôme d'étudée supérieures en aménagement et Urbanisme. Rabat Instituts.

Moukhliss, M., Taleb, A., Souabi, S., Ouallali, A., Spalevic, V. (2022). Groundwater quality forecasting using machine learning algorithms: Case Study Berrechid aquifer, central Morocco. Agriculture and Forestry, 68 (3), 35-56. doi:10.17707/AgricultForest.68.3.03.

Muller, S. D., Daoud-Bouattour, A., Fauquette, S., Bottollier-Curtet, M., Rifai, N., Robles, M., Saber, E.-R., El Madihi, M., Moukrim, S., & Rhazi, L. (2022). Holocene history of peatland communities of central Rif (Northern Morocco). Geobios, 70, 35-53. https://doi.org/10.1016/j.geobios.2021.12.001.

Mundy, J. (2015). Colonial Land Policies in Algeria and Morocco: Conflicts, Responses, and Land Transfers. International Journal of Middle East Studies, 47(3), 511-533.

Ouakhir, H., & El Ghachi, M. (2023). Temporal Dynamic of Soil Erosion and Rainfall Erosivity Within Srou River Basin (Middle Atlas / Morocco). American Journal of Mechanics and Applications, 11(1), 1–5. https://doi.org/10.11648/j.ajma.20231101.11.

Ouakhir, H., El Ghachi, M. El, Goumih, M., Ennaji, N., (2020). Fluvial Dynamic in Oued El Abid Basin: Monitoring and Quantification at an Upstream River Section in Bin El Ouidane Dam - 2016 / 2017- (Central High Atlas / Morocco). To cite this version: HAL Id: hal-02931241.

Ouallali, A,; Aassoumi, H.; Moukhchane, M.; Moumou, A.; Houssni, M.; Spalevic, V.; Keesstra, S. (2020). Sediment mobilization study on Cretaceous, Tertiary and Quaternary lithological formations of an external Rif catchment, Morocco, Hydrological Sciences Journal, 65:9, 1568-1582, https://doi.org/10.1080/02626667.2020.1755435

Pennell, C. R. (2000). Morocco since 1830: A History. C. Hurst & Co. Publishers.

Petrovic, S., Reed, A. (2023). Morocco. In: Petrovic, S. (eds) World Energy Handbook. Springer, Cham. https://doi.org/10.1007/978-3-031-31625-8_9

Rapport Mensuel d'Ensemble du Protectorat (R.M.E.P), (1924). Résidence générale de la république française au Maroc, juin p 3.

Sabri, E., Spalevic, V., Boukdir, A., Karaoui, I., Ouallali, A., Mincato, R.L., Sestras, P. (2022). Estimation of soil losses and reservoir sedimentation: A case study in Tillouguite Sub-basin (High Atlas-Morocco). Agriculture and Forestry, 68 (2): 207-220.

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

Simonnet, C. (2007). Agricultural Modernization in Colonial Morocco: The Doukkala Region from the 1920s to the 1950s. The Journal of North African Studies, 12(3), 293-311.

Tainter, J. A. (2013). The collapse of complex societies. Cambridge University Press.

The oral narration (2021). Revealed and disputed by Amghar of the Ait Werra tribe, 68 years old, dated May 21.

Tribunal coutumier des Ait Werra (1937). Registre n° 14, dossier n° 128, 4 juin.

Tribunal coutumier des Ait Werra (1951). Registre n° 28, dossier n° 265, 04 Juin.

Tribunal coutumier des Ait Werra (1951). Registre n° 28, dossier n° 378, 13 Aout.

Tribunal coutumier des Ait Werra (1954). Registre n° 32, dossier n° 26, 7 Janvier.

Tribunal de Béni Mellal (1924). Acte enregistré par N° 983, feuillet N° 84 du 8 septembre 1924 à la Mahkama du Caid de Béni Mellal. Registre N° 7.

Vaugien, J. (2014). Évolution d’une tribu berbère du Maroc central, les Aït Ouirra (Juillet 1950–janvier 1951) Introduction et notes de Gauthier Langlois, Villemoustaussou, 10 Mai 2014. P 84.

Zohary, D., Hopf, M., & Weiss, E. (2012). Domestication of plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press.

Ćirić, V., Prekop, N., Šeremešić, S., Vojnov, B., Pejić, B., Radovanović, D., Marinković, D. (2023): The implication of cation exchange capacity (CEC) assessment for soil quality management and improvement. Agriculture and Forestry, 69 (4): 113-133. doi:10.17707/AgricultForest.69.4.08

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THE IMPLICATION OF CATION EXCHANGE CAPACITY (CEC) ASSESSMENT FOR SOIL QUALITY MANAGEMENT AND IMPROVEMENT

SUMMARY

Soil consists of mineral and organic matter, possessing both chemical and physical, mineralogical and biological properties that provide a medium for plant growth and is therefore one of the most important conditions for agricultural production. On the other hand, soil cation exchange capacity (CEC) is a measure of the total capacity of soil to retain exchangeable cations and indicates the negative charge per unit mass of soil. Cation exchange capacity (CEC) is one of the many properties possessed by the soil, but its importance for the soil is multiple, which is particularly reflected in its ability to maintain soil fertility through binding and preventing the loss of cations from the soil due to binding to soil colloids. Cation exchange is a reversible reaction. CEC prevents the loss of nutrients and soil leaching, affects the availability of nutrients for plants, and serves as a basis for determining the required amounts of fertilizers. CEC in soil depends on the content of clay minerals and organic matter. Organic matter is the main source of CEC in sandy soils. Because of the different methods for estimating CEC, it is important to know the intended use of the data. For soil classification purposes, soil CEC is often measured at a standard pH value (pH 7). Since the value of CEC is a dependent variable property of the soil, its value depends on the content of clay minerals, organic matter, fertilization and pH value. Any change in soil properties leads to changes in CEC values, and therefore to changes in soil fertility.

Keywords: cation exchange capacity, CEC, agriculture, soil, organic matter, clay

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INTRODUCTION

Soil Cation Exchange Capacity is a very complex indicator that reflects a subset of soil physical and chemical properties of different individual values, and its interpretation can significantly improve agricultural production. This analysis is not included in the standard set of soil tests, but the values obtained can indicate the properties of the soil and the processes that are taking place. Of particular interest is the CEC monitoring of intensive vegetable production and seedling production. A correct interpretation of the CEC is therefore essential in order to take the appropriate measures to ensure successful production. Soils also have a distinct impact on human health (Brevik and Sauer, 2007), therefore soil contaminants constitute a known global problem and more knowledge is needed regarding their behavior as well as their pathways to humans (Abrahams, 2002; Semedo, 2017; Babec *et al.* 2020). The intensive use of land during the last decades has greatly influenced the change in its physical, chemical and biological properties (Šeremešić *et al.* 2017; Vojnov *et al.* 2019; Vojnov *et al.* 2020a; Kraamwinkel *et al.* 2021). In recent years, numerous authors have warned about the serious consequences of soil degradation that can result from its irrational use (Obalum *et al.* 2017; Semedo, 2017; Šeremešić *et al.* 2021a; Ferreira *et al.* 2022). Cation exchange capacity (CEC) is a soil property that allows it to bind positively charged ions (cations). In another words is a measure that expresses the ability of the soil to adsorb cations, and corresponding with plants capability of absorbing nutrients through the roots in ionic form (Jones and Kathrin, 2016).This is a very important property of the soil that affects the stability of structural aggregates, availability of nutrients for plants, regulates pH of the soil, as well as the reaction of the soil affected by fertilization and the addition of other ameliorants (Hazelton *et al.,* 2007). Clay minerals and components of soil organic matter contain a negative charge on their surface, which is why they adsorb and retain positively charged ions (cations) by electrostatic forces. This type of charge is of great importance for supplying plants with nutrients because many nutrients in the soil are found in the form of cations, such as magnesium (Mg^{2+}), potassium (K^{+}) and calcium (Ca^{2+}) and other elements. In general, soils that contain a higher degree of negative charge are more fertile soils because they have the ability to bind and retain larger amounts of cations (McKenzie *et al*., 2004). However, even on soils with a low capacity for cation exchange (CEC), high-yielding crops can be grown, if adequate and timely agrotechnical measures are applied. The major ions associated with soil CECs are the exchangeable cations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}) (Rayment *et al*., 1992) usually referred to base cations. In most cases, summing the analyzed base cations provides an adequate measure of CEC. However, as soils become more acidic, these cations are replaced by H^{+}, Al^{3+} and Mn^{2+} cations and common analytical methods will produce CEC values much higher than what is expected (McKenzie *et al.,* 2004). This cation exchange should be included when evaluating base cations and this measurement is then called effective cation exchange capacity (ECEC). Cation exchange capacity (CEC) of soil primarily results from clay and organic matter content. Soils contain, to a greater or lesser extent, silicate clay minerals that contain silicon in their composition. Each particle of clay consists of individual layers or sheets, since clay minerals in addition to silicon dioxide contain Al^{3+} ions, the total charge of clay minerals is negative (Miljković, 2005). The exchange of a silicon ion with aluminum in the crystal lattice is called an isomorphic exchange. Soil as a natural medium does not contain a negative charge due to the fact that the negative charge of clay minerals and colloidal particles of organic matter is in balance with the positive charge of cations (Brown *et al*., 2007), the negative charge of clay colloids associated with the isomorphic exchange of cations in the crystal lattice is considered is unchanged, regardless of changes in pH value.

IMPORTANCE OF CATION EXCHANGE CAPACITY (CEC) IN SOIL

Cation exchange capacity (CEC) is one of the many properties possessed by the soil, but its importance for the soil is multiple, which is particularly reflected in its ability to maintain soil fertility through binding and preventing the loss of cations from the soil due to binding to soil colloids. Cation exchange is a reversible soil characteristic (Nešić *et al.*, 2015). Preventing the loss of nutrients and soil leaching affects the availability of nutrients for plants and serves as a basis for determining the required amount of fertilizers. The interpretation of this property is based on the assumption that the greatest influence on CEC values in soil exerted the content of clay and organic matter (Hadžić *et al*., 1995). Organic matter is the main source of CEC in sandy soils (Johnson, 2002). In the soils of Vojvodina, the most common clay minerals belong to the illite group, and the cation exchange capacity is most accurately represented by the fact that 1 g of montmorillonite has an active surface of 700 m^2 with a negative charge, which is why it is able to bind huge amounts of nutrient cations and positively charged radicals from organic compounds, where are first retained, and then subjected to microbiological degradation (Nešić *et al*., 2015). The average content of humus in the soils of Vojvodina is 3% (Manojlović 2008). For this reason, it is important to study and define the physical and chemical properties of soil, as well as the content of organic matter and clay, as the most important factors of cation exchange, not only to preserve the current state, but also to improve and improve the state of soil fertility. Therefore, it is of crucial importance to study and understand the interrelationships of clay, humus content and their influence on CEC, because soil fertility and its ability to resist degrading changes depends on these properties. CEC is conventionally expressed in mec/100 g (Rengasamy and Churchman, 1999), which is numerically equal to centimoles of charge per kilogram of exchanger (cmol(+)/kg). The capacity of cation exchange in different types of soil varies in a wide interval from 1-2 cmol/kg to more than 100 cmol/kg of soil, most often CEC in soils is in the range of 25-35 cmol/kg of soil, while in the arable layer of loamy and clayey soils soils in Serbia that have a low humus content varies in the range of 20 to 40 cmol/kg of soil (Dugalić and Gajić 2012).

The content of $CaCO_3$ in the soil is very important, and it is especially important for CEC, because calcium in the soil affects the physical and chemical properties, in the form of Ca-humate, as a neutralizer or buffer. Soils that contain a large amount of kaolinite-type clay have a CEC in the range of 1-10 Meq/100 g, while soils that contain illite-type clay and smectite in the range of 25-100 Meq/100 g, organic matter has the capacity to exchange cations in which amounts to 250-400 Meq/100 g (Moore 1998). A high soil cation exchange capacity indicates that the soil has a high content of clay and organic matter, which further causes better water and nutrient retention than soils with a low CEC. Table 1. Shows the value of the cation exchange capacity defined according to (Hazelton et al., 2007).

Table 1. Cation exchange capacity value (Hazelton *et al*., 2007)

Value	**CEC cmol $_{(+)}$ kg^{-1}**
very low	<6
Low	6–12
Medium	12–25
High	25–40
very high	>40

THE EFFECT OF PH VALUE ON THE CATION EXCHANGE CAPACITY (CEC) IN THE SOIL

Because of the different methods for estimating CEC, it is important to know to use and interpret the obtained results. Therefore pH value of the soil, which further affects the availability of nutrients for the plant is considered of the most important soil properties that affects CEC. Cation exchange capacity can be expressed through different methods (Chapman, 1965; Sumner and Miller, 1996), however each of the methods expresses the assessed material and chemical conditions, especially the pH of the medium. Most methods involve saturating negative exchange sites with cations that form outer sphere complexes such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, NH^{4+}, then removing excess cations using an unbuffered solution and determining the amounts retained in the soil (Choo *et al*., 2016). An indirect method to calculate cation exchange capacity (CEC) is to sum exchangeable bases ($SB=Ca^{2+}+ Mg^{2+}+K^{+}+Na^{+}+NH^{4+}$) + exchangeable Al^{3+} and potential acidity ($H^{+}+Al^{3+}$) extracted with appropriate solutions (Donagemma *et al.*, 2011). For soil classification purposes, soil CEC is often measured at a standard pH value (pH 7). Examples are the ammonium acetate method of Schollenberger (1927) buffered at pH 7 and the barium chloride-triethanolamine method of Mehlich (1938) buffered at pH 8.2. Such CEC measurements can result in values that are very different from soil CEC values measured at the pH present in the field (effective CEC or CECE), especially in acidic soils with pH-dependent CEC. If a pH-buffered CEC measurement is required (e.g., for regulatory or soil classification purposes), ammonium acetate buffered to pH 7 is the recommended procedure, CEC determined by this method tends to be less than 3 cmolc/kg for sandy soils with low organic matter content up to more than

25 cmolc/kg for soils with a high content of certain types of clay minerals or organic matter. For an accurate measure of soil CEC in field conditions (Gillman, 1979, Gillman and Sumpter, 1986; Rhoades, 1982) proposed using a method using $BaCl^{2-}$. This method provides an estimate of the soil's capacity to retain base cations (CECb) under field conditions. To estimate the effective CEC (CECE), exchangeable Al is obtained by extraction with 1 M KCl (Bertsch and Bloom, 1996). Soil testing laboratories do not provide direct data for CEC, but data are obtained by recalculating CECsum, based on the amounts of extracted Ca^{2+}, Mg^{2+} and K^{+} obtained by laboratory methods (Ross *et al*. 2009). In soils that are rich in Na^{+}, the resulting extracted sodium is added to the calculations. If the soil pH is less than pH 6, the value of substitutional acidity is added to the sum of Ca^{2+}, Mg^{2+} and K^{+}, which further leads to the conclusion that significant amounts of exchangeable Al^{3+} ion may be present.

MUTUAL INFLUENCE OF CATION EXCHANGE CAPACITY AND FERTILIZERS

It is known that soil reaction depends on the amount and nature of the clay fraction, as well as on the presence and concentration of common cations and anions in the soil solution (Tessier *et al.,* 1999). The fine fraction of clay essentially ensures the regulation of the physic-chemical properties of the soil, and also has a significant role in water retention as well as the bioavailability of nutrients (Daoud *et al.,* 1992). The surface properties of soil components that have a significant role in the adsorption and retention of cations in the soil can be expressed through cation exchange capacity (CEC) or through the specific surface area of clay fractions (SS). The specific surface area of the clay fractions is determined using an organic molecule such as ethylene glycol monoethyl ether (EGME) which strongly adsorbs on the surface of the clay particles (Ciesielski *et al.,* 1997). CEC is measured by measuring the electrical conductivity of the surface components of the soil (Charlet, 1999). Knowledge of the capacity for cation exchange, the specific surface area of clay fractions, as well as the content of organic matter and the pH value of the soil are of great importance for the proper management and use of the soil, but also for the correct determination of the amount and use of fertilizers. Therefore, the addition of organic material is likely to increase the CEC of the soil over a period of time. On the other hand, soil CEC can also decrease over time, either naturally or by acidification caused by the addition of physiologically acidic fertilizers or decomposition of organic matter (McKenzie *et al*. 2004). Nitrogen mineral fertilizers used in agricultural production are key to achieving high yields, while knowledge of cation exchange capacity is necessary for quality soil management, it is especially important to point out that it is necessary to determine the content of organic nitrogen in the soil, which could be mineralized during vegetation period and, based on that, give a recommendation for fertilization with mineral nitrogen fertilizers, all with the aim of avoiding environmental pollution given that mineral forms of nitrogen in the soil, such as the NH^{4+} ion, can bind to the absorptive complex, while mineral forms of nitrogen in the form of NO^{3-} and NO^{2-} cannot bind, and if they are not absorbed by plants and microorganisms, they are washed into the groundwater (Kastori, 2005). Research results (Czarnecki *et al*., 2014) show that the long-term use of N, P, K fertilizers has significant effects on soil properties, during the study it was found that fertilization with N, P, K fertilizers has a significant effect on the reduction of soil pH. The decrease in the pH value of the surface layer of the soil when using mineral fertilizers can be attributed to the processes of nitrification and acidification stimulated by the continuous application of mineral fertilizers, as well as the release of H^{+} ions from the root system of plants (Liang *et al*., 2012). In the treatments where N fertilizer was applied, a slight difference in the pH value was observed compared to the initial value, while it was determined that the application of N, P, NP and NPK fertilizers significantly affects the pH value compared to the control in April when the analysis was done, however a significant difference in the reduction of pH value was observed when observing the control and treatments with N and P fertilizers in the month of August, while significant differences in the change in pH when applying only N fertilizer or only P fertilizer did not indicate significant differences in the change in pH value for November (Czarnecki *et al.*, 2014). The continuous application of ammonia fertilizers leads to a lowering of soil pH (Schwab *et al.*, 1990), which was proven by a study (Tsadilas *et al*., 2005), that indicated that the use of ammonia fertilizers has a greater effect on soil acidification than the application of nitrate fertilizers. Research conducted by Liu *et al*. (2007) proved that the application of ammonia fertilizers in leads to a lowering of the pH value of the soil from 4.51 to 4.07. The main mechanism of soil acidification with the use of ammonia fertilizers is the release of H^{+} ions in the nitrification process, as well as the subsequent leaching of NO^{3-} (Barak *et al*., 1997). The addition of fertilizers to the soil increases the concentration of the soil solution, which further results in increased plant nutrition, which is reflected in an increase in plant mass and yield, and also in an increase in the mass of crop residues returned to the soil (Haynes *et al.*, 1998). Research obtained by Raun *et al.* (1998) as well as (Halvorson *et al.*, 1999) showed an increase in the level of organic C in the soil with the application of N fertilizers. According to research Jagdish *et al.* (2011), the impact of long-term application of synthetic N fertilizers on soil organic matter has been questioned. The hypothesis that long-term application of N fertilizers leads to a decrease in soil organic carbon. Data from 135 studies of 114 long-term experiments located at 100 sites worldwide, over decades, under a range of land management and climate regimes, were used to quantify changes in soil organic carbon (SOC) and soil organic nitrogen (SON) Jagdish *et al*. (2011). Published data from a total of 917 and 580 observations for SOC and SON, representatively, from control (unfertilized or zero N) and N-fertilized treatments (synthetic, organic, and combined), were analyzed using SAS mixed model and meta-analysis. The results show a decrease of 7 to 16% in SOC and 7 to 11% in SON without fertilization with N fertilizers. In soils that received synthetic fertilizer N, the rate of SOM loss was reduced. Fertilizer time response ratio, which is based on changes in pairwise comparisons, showed an average increase of 8 and 12% for SOC and SON, respectively, after application of synthetic fertilizer N. Addition of organic matter (i.e., manure) increased SOM content, on average, by 37%. This paper shows a general decline in SOM for all long-term sites, with and without the application of synthetic N fertilizers. However, the analysis also shows that in addition to their role in improving crop productivity, synthetic N fertilizers significantly reduce the rate at which SOM is reduced in agricultural soils, worldwide. Research (Messiga *et al.,* 2013) also proved the increase in organic C content when increasing concentrations of N fertilizers were added to the soil. An increase in soil organic carbon content in the layer (0–15 cm) by 7.7% was observed in treatments where mineral fertilizers were added to the soil in contrast to the control (Liu *et al.,* 2005). Similar results were obtained in long-term studies where it was proven that low doses of N fertilizers had a weak effect on the increase of organic C in the soil compared to the control (1.14 vs. 0.03 mg ha^{-1} $year^{-1}$), while medium and high doses significantly influenced the increase in organic C (Corg) content, which ranged from 0.45 to 0.49 mg ha^{-1} $year^{-1}$ (Mazzoncini *et al.,* 2011). The long-term application of manure and mineral fertilizers, either alone or in combination with these fertilizers, has seen a significant increase in the content of organic matter in the soil, which leads to the conclusion that the application of mineral fertilizers has a stimulating effect on microbiological activity, whereby the organic matter of the soil decomposes, which leads to an increase in the availability of nutrients matter for plants, increase in above-ground mass and yield, and therefore to a greater amount of crop residues that are returned to the soil (Gong *et al.,* 2009). According to (Cakmak *et al.* (2010), the application of phosphorus fertilizers in a period of 40 years caused a decrease in soil pH and an increase in CEC. Research conducted by Schjonning *et al.* (1994) showed an increase in CEC values by 11% when using fertilizers compared to control plots. The application of nitrogen in the form of KAN fertilizer or in combination with the NPK complex raises the level of CEC in the soil, which can be explained by the colloidal retention of applied Ca^{2+}, NH^{4+} and K^{+} ions (Radulov *et al.*, 2011), and the content of organic C, which affects CEC soil, where a positive linear relationship between these two soil components was observed (Rashidi *et al*., 2008). Unlike other studies, research (Bationo *et al.,* 2007) found a negative linear relationship between pH value and organic C content, with two possible explanations for these negative observations: the first is that the accumulation of organic matter does not necessarily affect to a decrease in pH value, and another explanation is that other mechanisms affecting the change in pH are more dominant (Ritchie *et al.,* 1985). Specifically, the accumulation of undecomposed organic matter in soil rich in biological remains of symbiotic microorganisms and their products, as well as the application of ammonia fertilizers with the consequent leaching of nitrates, are involved in the accelerated acidification of agricultural soils (Bolan and Hedley, 2003). Fertilization of agricultural areas, in addition to affecting the pH and CEC values of the soil, also affects the increase in the content of other elements in the soil such as Cu and Zn, but other elements such as Pb, Cd, As, which belong to the group of heavy metals and can have a negative impact on the health of humans and animals that feed on plants grown on these lands (Atafar *et al*., 2010).

THE INFLUENCE OF SOIL MECHANICAL COMPOSITION ON CATION EXCHANGE CAPACITY (CEC) VALUES

It is well known that the content of clay minerals has a significant role in determining the physical, chemical and biological properties of the soil, but it also has a significant role in the formation of soil fertility potential, clay minerals as the smallest soil particles have a very important role in the adsorption and exchange of cations and anions with the soil solution, therefore it is of great importance to know the mineralogical composition of the soil and clay content in order to obtain a measure of soil fertility, determine the amount of necessary fertilizers and increase productivity (Hongling *et al*., 2019). The assumption is that sandy soils (especially soils with a large proportion of coarse sand) have a low content of clay, organic matter, for this reason also a low value of cation exchange capacity (CEC), which particularly affects their physical, chemical and mineralogical properties, and therefore the possibility of agricultural production (Fidalski *et al*., 2013). According to research (Soares and Alleoni, 2008; Adugna and Abegaz, 2015; Nešić *et al*., 2015) the content of clay and organic matter affects the CEC of the soil, while research (Saidian *et al*., 2016) made the opposite claim, indicating that the values CECs depend on the type of clay (smectite versus kaolinite) that was present in the soil, and not on the content of organic matter and the degree of its decomposition. Research (Hobley and Wilson, 2016) has shown that the mineralogical composition and the clay content in the soil have a great influence on the content of organic matter and its dynamics in the soil, and that pH-dependent charges play a significant role in increasing the CEC value in the soil associated with silanol and surface functional groups. Deprotonation of surface functional groups leads to the appearance of a negative charge, which leads to an increase in the cation exchange capacity (Sposito, 2008; Silva *et al*., 2012). In this way, positively charged iron and aluminum oxides bind to negatively charged functional groups of soil organic matter, building organo-mineral complexes. These complexes depend exclusively on the accumulation of organic matter as well as on the ability of the soil system to change its surface charge (Souza *et al*., 2020), thus preventing the mineralization of organic matter (Pishe *et al.*, 2011). The capacity for cation exchange is the most important chemical property of the soil and directly affects the loss of cations by leaching (Nešić *et al*., 2015). CEC values did not show a significant correlation ($p>0.05$) with Fe_2O_3 minerals, kaolinite. Kaolinite has a low CEC (~1 $cmol/kg^{-1}$) of clay, while Fe oxides have very low CEC values in soils due to high pH (>7.0), (Sposito, 2008). Clay minerals having a ratio of 2:1 was also not correlated with different CEC values due to the combination of low concentration (<50 g/kg^{-1}) and the presence of Al^{3+} ions in their interlayer which neutralizes the permanent negative charge of these minerals (Azevedo and Torrado, 2009). When we analyze the total organic carbon (TOC) and expressed data through the C:N ratio, as well as the crystallized forms of Fe and Al and the free forms of Fe and Al favored the correlation since the values of these four properties are very small in sandy and loamy soils. In addition, poorly crystalline forms of Fe and Al oxides have a higher affinity for binding with soil organic matter than free forms of Fe and Al (Cornell and Schvertmann, 2003). The colloid complex of the soil consists of humus and clay, the most important acidoids that are able to create bonds between oppositely charged ions (cations) through forces strong enough to provide protection against leaching, and also weak enough to allow adsorption through the plant's roots. This ability becomes more pronounced if the degree of dispersion is higher, that is, if the particles have smaller diameters. Research results (Nešić *et al.*, 2015) for different types of soil in Vojvodina, such as chernozem, fluvisol, semigley, humogley, soloncak and solonjec, showed that the cation exchange capacity depends on the clay fraction and humus content. A higher correlation coefficient between CEC and clay, compared to CEC and humus, indicates that clay content has a greater effect than humus content on the cation exchange capacity, and the results of these studies are shown in Table 2.

Table 2. Average values of clay, humus and CEC content depending on soil type (Nešić *et al.*, 2015).

Soil type	Clay %	Humus %	CEC (cmol/kg)
Chernozem	19,44	3,05	22,72
Fluvisol	25,61	2,69	22,41
Semigley	23,04	3,45	31,99
Humogley	40,75	3,25	30,97
Soloncak	21,88	2,90	26,61
Solonjec	26,20	2,78	40,06
Average value	26,15	3,02	29,13

INFLUENCE OF SOIL ORGANIC MATTER ON CATION EXCHANGE CAPACITY

The main source of organic matter in the soil is the remains of plants, animals and microorganisms, which are made of very complex organic substances such as: carbohydrates, proteins, fats, waxes, tannins, resins, pigments and organomineral complexes (Adugna and Abegaz, 2015). The importance of soil organic matter can perhaps best be described through the sentence that it represents the blood of the soil (Jungkunst, 2010). Its importance is also reflected in the characteristics that it serves as a reservoir of nutrients for plants by adsorbing nutrients, transforming them and protecting them from losses, which contributes to higher values of the capacity for cation exchange, and also affects the improvement of the physical properties of the soil, providing a medium for growth and the activity of microbes, significantly affects the buffering capacity of the soil when applying acids and bases, which makes it less susceptible to changes in pH value, and also affects the release of organic acids during decomposition, which in the soil affect the processes of wear and transformation of rock minerals (Alhassan *et al*., 2018; Tanimu and Liocks, 2013 and Totsche *et al.*, 2010). Studies on the effect of spatial variability of soil properties also indicate that soil organic matter and cation exchange capacity are strongly influenced by external factors such as agricultural production, soil management, as well as internal factors such as soil type and depth (Adugna and Abegaz, 2015). The content of organic matter characteristically decreases with increasing soil depth (Montes-Pulido *et al*., 2017), while the capacity for cation exchange varies depending on the depth and type of soil and is also correlated with the amount of separated organic matter in an individual soil depth (Tomašić *et al*., 2013). According to research (Topalović *et al.* 2022), organic matter enhances the olive tree productivity through positive effects on soil structure, water retention capacity and availability of some nutrients (limiting erosion, nitrogen leaching, phosphorus precipitation and iron inactivation). Soyergin *et al.* (2002) considered that the content of soil organic matter more than 1% being suitably for well growth. The level of total soil organic matter changes slowly and negative consequences for yield become visible only when the level of organic matter falls below 2% (Vojnov *et al*., 2020b; Šeremešić *et al.* 2021b), which can result in an irreversible process of degradation of all soil properties. Given that soil OM is the main reservoir of soil organic carbon (SOC) (Schmidt *et al*., 2011), it is necessary to investigate the fractions that are most prone to changes during land use (Ćirić, 2016; Šeremešić *et al.* 2020). Measuring and predicting the mechanisms responsible for soil organic matter conservation is very challenging due to the long periods required to detect changes, as well as the complexity of the physicochemical properties responsible for soil organic C stabilization (Van der Voort *et al*., 2016; Harden *et al*., 2018). In addition, the biotic activity of plants and microorganisms, as well as the physical and chemical properties of the soil, significantly influence the dynamics of organic matter in the soil (Torn *et al*., 1997; Schmidt *et al*., 2011; Doetterl *et al*., 2015; Rasmussen *et al*., 2018). A soil property used to determine variation in soil organic matter content and stability is percent clay (Coleman and Jenkinson, 1996; Wieder *et al*., 2015). In principle, the clay content controls the stabilization of organic matter through the sorption of organic molecules on mineral surfaces <2 μm in size, as well as their occlusion into aggregates (Oades, 1988; Eusterhues *et al*., 2003; Lutzov *et al*., 2006). Structural soils were found to contain more organic matter than coarser textured soils (Rasmusen *et al.*, 2018). Although clay content for the purposes of assessing organic matter content is widely used in research due to ease of measurement, new research shows that this approach can simplify and inefficiently depict soil mechanisms that affect the preservation of organic matter (Bailey *et al*., 2018; Rasmussen *et al.*, 2018). Clay minerals represent a class of minerals whose surfaces come into contact with soil organic matter (Kaiser and Guggenberger, 2000; Vogel *et al.*, 2014). Research (Farrar and Coleman, 1967) suggests that the clay content does not provide sufficient information about the surface of the soil that can be available for the sorption of organic matter. Accordingly, other soil properties that affect the sorptive capacity of the soil can give a clearer picture of the mechanisms that affect the preservation and stabilization of organic matter in the soil (Bailey *et al.,* 2018). The sorptive capacity of soil surfaces represents the effective capacity for cation exchange (CEC ef.) and it represents the total amount of exchangeable cations that the soil can adsorb on its surfaces, at the pH value present in the field, at the low pH value present, mostly permanent charges of clay type 2:1 adsorb exchangeable cations, while at higher pH values positive ions are adsorbed on surfaces with variable charges such as 1:1 type clay minerals, allophane, organic matter and Fe and Al oxides (Weil and Brady, 2016). The effective capacity for cation exchange can represent a direct measure of the sorption of organic matter to adsorptive soil surfaces at existing pH conditions, whereby polyvalent metal cations such as Al^{3+} and Ca^{2+} can play an important role in stabilizing organic matter, by binding organic compounds to mineral surfaces through exchangeable bridges and ionic bonds (Oades, 1988; Rasmussen *et al.,* 2018; Rovley *et al.,* 2018). Metal cations are of great importance in the binding of organic compounds in the soil, and the binding and stabilization of organic matter depends on the hydration shell and the valence of the cation, which is proven by chemical modeling that shows that bridges of exchangeable Ca^{2+} ions are more stable than bridges with exchangeable monovalent cations, because the radius ratio charging and hydration of Ca^{2+} ions enables a balanced repulsion between negatively charged particles in the soil (Sutton *et al*., 2005; Iskrenova-Čukova *et al*., 2010). The complexation of organic molecules with methane cations that are present on the surface of minerals through ionic bonds mainly refers to the displacement from the hydration shell of cations, the ionic potential, as well as the type of organic molecules present in the soil solution (Rowley *et al.,* 2018). In acidic soils, it was observed that the Al^{3+} ion plays a very important role in the complexation of organic compounds and the mineral surface, while the Fe^{3+} ion forms insoluble precipitates in the present pH conditions and is rarely found in the soil as a free ion, and the Ca^{2+} ion is weakly polarized and tends to form ionic bonds with ligands containing oxygen (O), such as carboxylic acids, and for this reason have the ability to form complexes with organic compounds (Sposito, 2008). Monovalent cations such as the Na^{+} ion do not form ionic bonds with organic ligands, while the K^{+} ion participates in complexes found in the interlayers of certain phyllosilicates (Rowley *et al*., 2018). In addition to all of the above, metal cations can form organo-mineral complexes in the soil by linking several organic compounds together, which have been observed to cause aggregation and thus affect the physical protection of soil organic matter (Kunhi Mouvencheri *et al.,* 2012).

PREDICTION OF CEC WITH PEDOTRANSFER FUNCTIONS

The soil pedotransfer function (PTFs) is used to obtain information on the most important soil properties, which are difficult to obtain (time or financially).

The pedotransfer function is defined as a statistical model for predicting physical (bulk mass, water-air regime, etc.) and chemical (cation exchange capacity) soil properties (Kumar and Gurung, 2002). Pedotransfer soil functions are also applied in soil studies to assess values that are difficult to measure in the field (Minasny and Hartemink, 2011). The soil pedotransfer function is obtained from available parameters from soil reserves, such as organic carbon and clay content. It is very important to get an equation that will show the parameters with certainty (Benites, 2007). The consistency of PTFs mainly depends on the number of samples and the range of input parameters (Chirico *et al.*, 2010). Pedotransfer functions for CEC are often developed from a combination of different properties such as clay content, organic matter content and hygroscopic water content (Arthur 2017; Krogh *et al*., 2000; McBratney *et al.*, 2002; Olorunfemi *et al*., 2016.). Research (Krogh *et al*. 2000) showed that PTFs based on clay and organic matter content in illite-dominated samples reliably explained 90% of CEC variability. Similar to research (McBratney *et al*. 2002), it was demonstrated that PTFs based on clay and OM content explained 73% of CEC variability. However, due to spatial variations in clay mineralogy and organic matter composition, it is necessary to develop different PTFs for different regions and soil types (Seybold *et al*. 2005). Regression and fuzzy logic are common methods used in PTF development (Ostvari *et al*. 2015a; Ostovari *et al.* 2019). In statistics, multiple linear regression (MLR) is a linear approach to modeling the relationship between a dependent variable and one or more independent variables (Herbst *et al*. 2002). The regression tree (RT) is a well-known method used in environmental science (Ostovari *et al.* 2019; Pachepsky and Rawls 2006; Pachepsky and Schaap 2004). This method represents data collection techniques that use qualitative and quantitative variables as independent variables. In the (RT) method, independent variables are introduced which are then selected by the software as effective variables (with high correlation) and divided into two groups according to the order of priority where by a tree-like structure is created, where in the first node there is a certain property that is divided into two groups/nodes by the variable that has the highest correlation with it. The best regression tree results when the variable chosen to create the greatest homogeneity in the new nodes is well correlated (Ostovari *et al.* 2019). In the research conducted by Keshavarzi, Sarmadian and Labbafi (2011), models were developed to predict CEC using MFIS and ANN, where data on clay content and % OM were used as variables in both systems. The results of that research showed that MFIS had a higher efficiency than ANN in predicting CEC. According to the research conducted by (Ostovari *et al*. 2015b) where the MFSI and RT method were compared in the estimation of CEC in the field, different soil properties including density, sand, dust and clay content were used as input variables in both methods, the results were showed that (RT) was more effective than MFSI in estimating CEC. Assessment of CEC in soils in Semnana province (Iran) using ANN, MLR and fuzzy logic methods was performed on the basis of 200 soil samples, a regression model and non-specific rules were developed (Emamgolizadeh and Ghorbani, 2015). The results of these studies confirmed that the ANN method had better CEC prediction results than the MLR method and fuzzy logic. In separate studies conducted by (Ghorbani *et al.* 2015) and (Seyed-Mohammadi *et al*. 2016) where the ANN, MLR and fuzzy logic methods were compared, in the evaluation of CEC values, it was also found that the ANN method had better estimation results from MLR and fuzzy logic methods. In research conducted by (Nikseresht *et al*. 2019), a total of 100 soil samples collected across the USA were used, with the samples being divided into two groups, where 75 samples served as a calibration set for the MLR and RT models as well as to form fuzzy logic functions, the remaining 25 samples were used to validate the performance evaluation of MLR, RT and MFSI for the estimation of CEC values. For the development of PTFs using MLR, RT and MFSI methods, easily measurable soil properties such as soil particle size, organic matter content, volumetric mass, etc. were used. To begin with, the relationship between easily measurable soil properties and CEC was investigated, where the following linear equation CEC=aX + b as well as a non-linear equation in the form of CEC=a log(X) + b was used for this purpose. Where X is an easily measurable property of the soil, a is the slope of the linear line and b is the intercept. The same easily measurable soil properties were used in the regression methods (MLR and RT) and were also used as input variables in the MFSI to predict CEC. To prevent multicollinearity given the high correlation between soil particle size (r=0.85, % clay with % dust and r=-0.064 between % clay with % sand), the mean geometric diameter of the particles (dg) and the fractal dimension (D) obtained from soil particle size were applied to develop PTFs. These two values were calculated according to the proposed equations of Shirazi and Boersma (1984) and Sepaskhah and Tafteh (2013) as follows:

$$dg = exp\ [\ 0,1\ (P_{sa} * In(1,025) + P_{si} * In(\ 0,026) + P_{cl} * In(0,001))]$$

$$D = 3 - 0,118\ \Big[\ -Ln\frac{Pcl}{100} + \left(\frac{Psi+Psa}{100}\right)\Big]$$

where P_{sa}, P_{si} and P_{cl} are % sand, % dust and % clay.

Research results (Nikseresht *et al*. 2019) showed that CEC has a positive significant correlation with % OM (r=0.68; p<0.01) and D (r=0.68; p<0.01). CEC also had a negative significant correlation with BD (r=-0.35; p<0.05) and dg (r=-0.52; p<0.05) Previous research conducted by (Ersahin *et al*. 2006) and (Baiat *et al.* 2014) also showed a positive correlation between CEC and D. In many previous studies conducted such as Keshavarzi, Sarmadian and Labbafi (2011), Shirani and Rafienejad (2011), and Memarian-Fard and Beigi (2009) and Ghorbani, Kashi, and Moghadas (2015), a highly significant correlation was found between CEC content and OM. Research (Ostovari *et al*., 2015) shows that in the range from 2.05 to 2.60 for D and 0% to 2% for OM, CEC has a minimum value, and with an increase in D and % OM, CEC values increase gradually. When it comes to soils with a high $CaCO_3$ content (carbonate soils), the assessment of CEC is difficult and expensive, although it can be measured by laboratory analysis (Carpena *et al.* 1972). There are various PTF models for estimating CEC values, related to basic physical and chemical soil characteristics, but PTF models that estimate CEC values on carbonate soils are poorly developed (Mahmoud *et al*. 2016, Khaledian *et al*. 2017). Research conducted by (Fattah *et al*. 2021) aimed to model PTFs that will predict CEC values in carbonate soils with high pH and low OM content, and for wide ranges in clay content, at for which they used statistical models based on different efficiency criteria (RMSE, AAPE, EF and PIME), whereby models were used:

$$RMSE = \sqrt{\frac{1}{(n-df)}\sum_{i=1}^{n}(O_i - P_i)^2}$$

$$AAPE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{O_i - P_i}{o_i}\right| 100$$

$$EF = 1 - \frac{\sum_{i=1}^{n}(Oi - P_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}$$

where RMSE-root mean square error, AAPE-average absolute error in percentage and EF-efficiency coefficient of Nash-Sutcliffe model, Oi-observed values, Pi-predicted values, n-number of observations, O-mean value of observed values.

Research results (Fattah *et al*. 2021) showed that in carbonate soils with low OM content and high pH value, CEC values are related to the content of clay minerals, which are a source of negative charge.

CONCLUSIONS

Knowing the value of CEC and humus content are the basis for the calculating required amount of fertilizers, while their monitoring can help in a prevention of nutrient leaching and ensure environmental protection. This study showed that although there are the trash holds and different level of CEC, this soil property has unique characteristics for single soil site. However, in many agronomic soil studies CEC has not received significant attention and recognition. In the future, soil analysis will benefit from accurate and extended data evaluation, as well as cost-effective models that can provide comprehensive insight between soil chemical, physical and microbiological properties. Given that CEC has a unique ability to encompass many soil properties knowing this value could help in defining cropping technology for stable yields and soil quality preservation. Therefore, farmers and practitioners should consider using CEC as a decision making tool that could be crucial for agricultural production in the future.

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REFERENCES

Abrahams, P. W. (2002). Soils their implications to human health, Sci. Total Environ., 291, 1–32.

Adugna, A., and Abegaz, A. (2015). Effects of soil depth on the dynamics of selected soil properties among the high lands resources of Northeast Wollega, Ethiopia: are these sign of degradation? Solid Earth Discussions, 7(3), 2011–2035.

Alhassan, I., Gashua, A. G., Dogo, S., and Sani, M. (2018). Physical Properties and Organic Matter content of the soils of Bade in Yobe State, Nigeria. International Journal of Agriculture, Environment and Food Sciences, 2(4), 160–163

Arthur, E. (2017). Rapid estimation of cation exchange capacity from soil water content.

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam M., and Mahvi A., H. (2010). Effect of fertilizer application on soil heavy metal concentration, Environ. Monit. Assess., 160, 83–89.

Azevedo, A, Vidal-Torrado P. (2009). Esmectita, vermiculita, minerais com hidróxi entre camadas e clorita. In: Melo VF, Alleoni LRF, editores. Química e Mineralogia do Solo - Parte I: Conceitos Básicos. Viçosa, MG: Sociedade Brasileira de Ciência do Solo. 381-426.

Bailey, V. L., Bond-Lamberty, B., DeAngelis, K., Grandy, A. S., Hawkes, C. V., Heckman, K. (2018). Soil carbon cycling proxies understanding their critical role in predicting climate change feedbacks. Glob. Change Biol. 24, 895–905.

Barak, P., Jobe, B. O., Krueger, A. R., Peterson, L. A., Laird, D. A. (1997). Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin, Plant Soil, 197, 61–69, 1997.

Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B., and Kimetu J. (2007). Soil organic carbon dynamics, functions and management in West African agro-ecosystems, Agr. Syst., 94, 13–25.

Benites, V. M., Machado, P. L. O. A., Fidalgo, E. C. C., Coelho, M. R., Madari, B. E. (2007). Pedotransfer functions for estimating soil bulk density from existing soil survey reports in Brazil. Geoderma, 139(1-2), 90–97.

Bertsch, P. M., Bloom, P. R. (1996). Aluminum. In it'Iethods of soil anal iris part 3: Chemical methods (SSSA Book Series No. 5). ed. D. L. Sparks. Madison, Wisc.: SSSA/ASA.

Bolan, N. S., Hedley, M. J. (2003). Role of Carbon, Nitrogen, and Sulfur Cycles in Soil Acidification, in: Handbook of soil acidity, edited by: Rengel, Z., Marcel Dekker, Inc., New York.

Brevik, E. C., Sauer, T. J. (2007). The past, present, and future of soils and human health studies, SOIL Discuss., 1 51-80.

Brown, K., Lemon, J. (2007). Cornell University Cooperative Extension (CUCE). Cation Exchange Capacity (CEC). Agronomy Fact Sheet Series # 22. Department of Crop and Soil Sciences, College of Agriculture and Life Sciences, Cornell University.

Cakmak, D., Saljnikov, E., Mrvic, V., Jakovljevic, M., Marjanovic, Z., Sikiric, B., Maksimovic, S. (2010). Soil properties and trace elements contents following 40 Years of phosphate fertilization. J. Environ. Qual., 39, 541–547. Capacity and permanent wilting point using UNSODA and HYPRES datasets. Arid Land Research and Management

Chapman, H. D. (1965). Cation-exchange capacity. In: Black CA, editor. Method of soil analysis: Chemical and microbiological properties. Part 2. Madison: American Society Agronomy; p. 891-900.

Charlet, I., and Schlegel, M. l. (1999). La capacité d'échange des sols. Structures et charges à l'interface eau/particule, Compte Rendu d'Académie d'Agriculture. Paris, France, 85(2), 7-24.

Chirico, G.B., Medina, H., Romano, N. (2010). Functional evaluation of PTF prediction uncertainty: An application at hillslope scale. Geoderma 155:193–202.

Choo, K. Y., Bai, K. (2016). The effect of the mineralogical composition of various bentonites on CEC values determined by three different analytical methods. Appl Clay Sci;126:153-9.

Ciesielski, H., Sterckemann, T. (1997). Determination of exchange capacity and exchangeable cations in soils by means of cobalt hexamine trichloride. Effects of experimental conditions, Agronomie, 17, 1-7.

Ćirić, V., Belić M., Nešić Lj., Šeremešić S., Pejić B., Bezdan A., Manojlović M. (2016). The sensitivity of water extractable soil organic carbon fractions to land use in three soil types, Archives of Agronomy and Soil Science, 62(12), 1654-1664.

Coleman, K., Jenkinson, D. (1996). "RothC-26.3-A Model for the turnover of carbon in soil," in Evaluation of Soil Organic Matter Models, eds Smith J. U., Smith P., Powlson D. S., (Cham: Springer), 237–246.

Cornell, R. M., Schwertmann, U. (2003). The iron oxides. 3rd ed. Weinheim: VHC.

Czarnecki, S., Düring, R.-A. (2014). Institute of Soil Science and Soil Conservation, Justus Liebig University, Giessen, Germany. Influence of long-term mineral fertilization on metal contents and properties of soil samples taken from different regions.

Daoud, Y., Robert, M. (1992). Influence of particle size and clay organisation on hydraulic conductivity and moisture retention of clays from saline soils. Applied Clay Science, 6, 293-299.

Doetterl, S., Stevens, A., Six, J., Merckx, R., Van Oost, K., Pinto, M. C. (2015). Soil carbon storage controlled by interactions between geochemistry and climate. Nat. Geosci. 8, 780–783.

Donagemma, G. K., Campos, D. V. B., Calderano, S. B., Teixeira, W. G., Viana J. H. M. (2011). Manual de métodos de análise do solo. 2. ed. rev. Rio de Janeiro: Embrapa solo.

Dugalić, J., Gajić, G., Boško, A. (2012). Pedologija. Faculty of Agronomy, Čačak

Emamgolizadeh, S., Bateni, S., Shahsavani, D., Ashrafi, T., Ghorbani, H. (2015). Estimation of soil cation exchange capacity using Genetic Expression Programming (GEP) and Multivariate Adaptive Regression Splines (MARS), Journal of Hydrology Volume 529, Part 3, Pages 1590-1600.

Ersahin, S., Gunal, H., Kutlu, T., Yetgin, B., Coban, S. (2006). Estimating specific surface area and cation exchange capacity in soils using fractal dimension of particle-size distribution. Geoderma, 136, 588–597. Eur. J. Soil Sci. 68(3), 365–373.

Eusterhues, K., Rumpel, C., Kleber, M., Kögel-Knabner, I. (2003). Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Organ. Geochem. 34, 1591–1600.

Farrar, D. M., Coleman, J. D. (1967). The correlation of surface area with other properties of nineteen british clay soils. J. Soil Sci. 18, 118–124.

Fattah, M. A., Karim, K. H. (2021). Perfformance of linear models in predicting cation exchange capacity calcareus soils. Iraqi Journal of Agricultural Sciences. 52(6):1489-1497.

Ferreira, C. S., Seifollahi-Aghmiuni, S., Destouni, G., Ghajarnia, N., & Kalantari, Z. (2022). Soil degradation in the European Mediterranean region: Processes, status and consequences. Science of the Total Environment, 805, 150106.

Fidalski, J., Tormena, C. A., Alves, S. J., Auler, P. A. M. (2013). Influência das frações de areia na retenção e disponibilidade de água em solos das formações Caiuá e Paranavaí. Rev Bras Cienc Solo. 2013 (37):613-21.

Ghorbani, H., Kashi H., Moghadas N. (2015). Estimation of soil cation exchange capacity using multiple regression, artificial neural networks, and adaptive neuro-fuzzy inference system models in Golestan Province, Iran. Communications in Soil Science and Plant Analysis 46(6):763–80.

Gillman, G. P. (1979). A proposed method for the measurement of exchange properties of highly weathered soils. Aust. J., Soil. Res. 17:129-139.

Gillman, G. P., Sumpter E. A. (1986). Modification to the compulsive exchange method for measuring exchange characteristics of soils. Aust. J. Soil Res. 24:6.

Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y. (2009). Long-term manure and fertilizer effects on soil organic matter fractions and microbes under some wheat–maize cropping system in northern China, Geoderma, 149, 318–324.

Hadžić, V., Molnar, I., Nešić, Lj., Kurjački, I. (1995). Uticaj meliorativnih mera na kapacitet adsorpcije solonjeca. Savremena poljoprivreda 43(4): 5-9.

Halvorson, A. D., Reule, C. A., Follett, R. F. (1999). Nitrogen Fertilization Effects on Soil Carbon and Nitrogen in a Dryland Cropping System, Soil Sci. Soc. Am. J., 63, 912–917.

Harden, J. W., Hugelius, G., Ahlström, A., Blankinship, J. C., Bond-Lambertym B., Lawrence C. R., (2018). Networking our science to characterize the state, vulnerabilities, and management opportunities of soil organic matter. Glob. Change Biol. 24, 705–718.

Hazelton P. A., Murphy, B. W., (2007). 'Interpreting Soil Test Results What Do All The Numbers Mean' CSIRO Publishing: Melbourne

Herbst, M., Diekkrüger, B. (2002). The influence of the spatial structure of soil properties on water balance modeling in a microscale catchment. Physics and Chemistry of the Earth, Parts A/B/C, 27(9-10), 701–710.

Hongling, B., Dong, L., Peng, Y., Xiang, Z., Hongmei, L., Peixin, D. (2019). Ethylene glycol monoethyl ether (EGME) adsorption by organic matter (OM)-clay complexes: Dependence on the OM Type. Applied Clay Science, 168, 340-347.

Iskrenova-Tchoukova, E., Kalinichev, A. G., Kirkpatrick, R. J. (2010). Metal cation complexation with natural organic matter in aqueous solutions: molecular dynamics simulations and potentials of mean force. Langmuir 26, 15909–15919.

Jagdish, K. L. C., Kesava, C. R., Padre, A. T., Van Kessel, C. (2011). Role of Nitrogen Fertilization in Sustaining Organic Matter in Cultivated Soils, Journal of Environmental Quality, Volume 40, Issue 6, Pages 1756-1766.

Johnson, C. E. (2002). Cation exchange properties of acid forest soils of the north eastern USA. European Journal of Soil Science. 53: 271- 282.
Jones, C., Kathrin, O. R. (2016). Plant Nutrition and Soil Fertility. Montana, State University. 1–12.
Jungkunst,H. F. (2010). Arctic thaw. Nature Geoscience, 3(5), 306–307.
Kaiser, K., Guggenberger, G. (2000). The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organ. Geochem. 31, 711–725.
Kastori, R. (2005). Fiziologija biljaka, Naučni institut za ratarstvo i povrtarstvo Novi Sad.
Kaur, R., Kumar, S., Gurung, H. P. (2002). Australian Journal of Soil Research, 40(5), 847.
Keshavarzi, A., Sarmadian, F., Labbafi, R., & Vandechali, M. R. (2011). Modeling of soil cation exchange capacity based on fuzzy table look-up scheme and artificial neural network approach. Modern Applied Science, 5(1), 153.
Khaledian, Y., Kiani, F., Ebrahimi, S., Brevik, E. C., & Aitkenhead- Peterson, J. (2017). Assessment and monitoring of soil degradation during land use change using multivariate analysis. Land Degradation & Development, 28(1), 128-141.
Kraamwinkel, C. T., Beaulieu, A., Dias, T., & Howison, R. A. (2021). Planetary limits to soil degradation. Communications Earth & Environment, 2(1), 249.
Krogh, L., Breuning-madsen, H., Greve, M.H. (2000). Cation exchange capacity pedotransfer functions for Danish soils. Acta Agriculturae Scandinavica, Section B — Soil & Plant. Science 50(1), 1–12.
Kunhi Mouvenchery, Y., Kucerík, J., Diehl, D., Schaumann, G. E. (2012). Cation-mediated cross-linking in natural organic matter: a review. Rev. Environ. Sci. Bio Technol. 11, 41–54.
Liang, Q., Chen, H., Gong, Y., Fan, M., Yang, H., Lal, R., Kuzyakov, Y. (2012). Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain, Nutr. Cycl. Agroecosyst., 92, 21–33.
Liu, J., Duan, C. Q., Zhu, Y. N., Zhang, X. H., Wang, C. X. (2007). Effect of chemical fertilizers on the fractionation of Cu, Cr and Ni in contaminated soil, Environ. Geol., 52, 1601–1606.
Liu, X., Liu, J., Xing, B., Herbert, S. J., Meng, K., Han, X., Zhang, X. (2005). Effects of long-term continuous cropping, tillage, and fertilization on soil organic carbon and nitrogen of Black Soils in China, Commun. Soil Sci. Plan., 36, 1229–1239.
Lützow, M. V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. Eur. J. Soil Sci. 57, 426–445.
Manojlović, M. (2008). Đubrenje u održivoj poljoprivredi, Faculty of Agriculture, Novi Sad.
Mazzoncini, M., Sapkota, T. B., Barberi, P., Antichi, D., Risaliti, R. (2011). Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content, Soil Till. Res., 114, 165–174.
McBratney, A. B., Minasny, B., Cattle, S. R., Vervoort, R. W. (2002). From Pedotransfer Functions to Soil Inference Systems. Geoderma, 109, 41-73.
McKenzie, N. J., Jacquier, D. J., Isbell, R. F., Brown, K. L., (2004). 'Australian Soils and Landscapes An Illustrated Compendium' CSIRO Publishing: Collingwood, Victoria.

Mehlich, A. (1938). Use of triethanolamine acetate-barium hydroxide buffer for the determination of some base exchange properties and lime requirement of soil. Soil Sci. Soc. Am. Proc. 29:374-378.

Memarian-Fard, M., Beigi, H. (2009). Comparison of artificial neural network and regression pedotransfer functions 23(4): 90-99.

Messiga, A. J., Ziadi, N., Belanger, G., Morel, C. (2013). Soil nutrients and other major properties in grassland fertilizes with nitrogen and phosphorus, Soil Sci. Soc. Am. J., 77, 643–652.

Miljković, N. (2005). Univerzitet u Novom Sadu, Poljoprivredni fakultet, departman za uređenje voda, knjiga meliorativna pedologija.

Minasny, B., Hartemink, A. E. (2011). Predicting soil properties in the tropics. Earth-Science Reviews, 106, 52-62.

Montes-Pulido, Carmen Rosa, José Joaquín Ramos-Miras, San José-Wery, and Ana María (2017). Estimation of soil organic carbon (SOC) at different soil depths and soil use in the Sumapaz paramo, Cundinamarca-Colombia. Acta Agronómica, 66(1): 95-101.

Moore, G. (1998). Soil Guide: a handbook for understanding and managing agricultural soils. Department of Agriculture, Western Australia Bulletin. 4343.

Nešić, L., Vasin, J., Belić, M., Ćirić, V., Gligorijević, J., Milunović, K., Sekulić, P. (2015). The colloid fraction and cation-exchange capacity in the soils of Vojvodina, Serbia. Ratar Povrt. 52:18-23.

Nikseresht, F., Honarbakhsh, A., Ostovari, Y., Afzali, S. F. (2019). Model Development to Predict CEC Using the Intelligence Data Mining Approaches. Communications in Soil Science and Plant Analysis, 1–12.

Oades, J. M. (1988). The retention of organic matter in soils. Biogeochemistry 5, 35–70

Obalum, S.E.; Chibuike, G.U.; Peth, S.; Ouyang, Y. (2017). Soil organic matter as sole indicator of soil degradation. Environ. Monit. Assess. 189, 1-19.

of Serbia. Agriculture and Forestry, 67 (3): 177-189

olives grown in the Marmara region. Acta Horticulturae, 586, 375-379.

Olorunfemi, I. E., J. T. Fasinmirin., Ojo, A. S. (2016). Modeling cation exchange capacity and soil water holding capacity from basic soil properties. Eurasian Soil Sci. 4:266–274.

Ostovari, Y., Asgari, K. and Cornelis, W. (2015a). Performance evaluation of pedotransfer functions to predict field capacity and permanent wilting point using UNSODA and HYPRES datasets. Arid Land Research and Management, 29(4),383-398.

Ostovari, Y., Asgari, K., Cornelis, W., & Beigi-Harchegani, H. (2015b). Simple methods for estimating field capacity using Mamdani inference system and regression tree. Archives of Agronomy and Soil Science, 61(6), 851-864.

Ostovari, Yaser, Shoja Ghorbani-Dashtaki, Lalit Kumar, and Farzin Shabani. (2019). Soil erodibility and its prediction in semi-arid regions. Archives of Agronomy and Soil Science, 65(12):1688-1703.

Pachepsky, Y. A., Rawls, W. J. (2006). Hydrogeology and pedotransfer function. Geoderma 131:308–16.

Pachepsky, Y., Schaap, M. G. (2004). Data mining and exploration techniques. In Developments in soil science. 30: 21-32.

Pishe, M. Z., Khormali, F., Shariari, A. (2011). The relationship between specific surface área and soil organic carbon in loess-derived soil of Northern Iran. Dyn Soil Dyn Plant. 2011;5:87-9. prediction models for calcareous soils. J. Plant Nutr. Soil Sci. 180 (3), 347–354.

Radulov, I., Berbecea, A., Sala, F., Crista, F., Lato, A. (2011). Mineral fertilization influence on soil pH, cationic exchange capacity and nutrient content, Res. J. Agric. Sci., 43, 160–165.

Rashidi, M., Seilsepour, M. (2008). Modelling of soil cation exchange capacity based on soil organic carbon, ARPN J. Agr. Bio. Sci., 3, 41–45.

Rasmussen, C., Heckman, K., Wieder, W. R., Keiluweit, M., Lawrence, C. R., Berhe, A. A. (2018). Beyond clay: towards an improved set of variables for predicting soil organic matter content. Biogeochemistry 137, 297–306.

Raun, W. R., Johnson, G. V., Phillips, S. B., Westerman, R. L. (1998). Effect of long-term N fertilization on soil organic C and total N in continuous wheat under conventional tillage in Oklahoma, Soil Till. Res., 47, 323–330.

Rayment, G. E., Higginson, F. R. (1992). Electrical Conductivity. In 'Australian Laboratory Handbook of Soil and Water Chemical Methods' Inkata Press: Melbourne regions. Archives of Agronomy and Soil Science 65:1688–703.

Rengasamy, P., Churchman, G. J. (1999). Cation Exchange Capacity, Exchangeable Cations and Sodicity. In 'Soil Analysis an Interpretation Manual' (Eds KI Peverill, LA Sparrow and DJ Reuter). CSIRO: Melbourne.

Rhoades, J. D. (1982). Cation exchange capacity. In: A.L. Page (ed.) Methods of soil analysis. Part 2: Chemical and microbiological properties (2nd ed.) Agronomy 9:149-15

Ritchie, G. S. P. and Dolling, P. J.: The role of organic matter in soil acidification, Aust. J. Soil Res., 23, 569–576.

Rowley, M. C., Grand, S., Verrecchia, É. P. (2018). Calcium-mediated stabilisation of soil organic carbon. Biogeochemistry 137, 27–49.

Saidian, M., Godinez, L. J., Prasad, M. (2016). Effect of clay and organic matter on nitrogen adsorption specific surface area and cation exchange capacity in shales (Mudrocks). J Nat Gas Sci Eng. 2016;33:1095-106.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A. (2011). Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Schollenberger, C. J. (1927). Exchangeable hydrogen and soil reaction. Sci. 35:552-553

Schwab, A. P., Owensby, C. E., Kulyingyong, S. (1990). Changes in soil chemical properties due to 40 years of fertilization, Soil Sci., 149(1):35-43.

Semedo, H. (2017). Only 60 years of farming left if soil degradation continues, https://www.scientificamerican.com/article/only-60-years-of-farming-leftif-soil degradation-continues/.

Sepaskhah, A. R., Tafteh, A. (2013). Pedotransfer function for estimation of soil-specific surface area using soil. 59(1), 93-103.

Šeremešić, S. I., Nešić, L. M., Ćirić, V. I., Vasin, J. R., Đalović, I. G., Marinković, J. B., & Vojnov, B. Đ. (2020). Soil organic carbon fractions in different land use systems of Chernozem soil. Zbornik Matice srpske za prirodne nauke, (138), 31-39.

Šeremešić, S., Dolijanović, Ž., Tomaš Simin, M., Vojnov, B., & Glavaš Trbić, D. (2021a). The Future We Want: Sustainable Development Goals Accomplishment with Organic Agriculture. Problemy Ekorozwoju, 16(2), 171-180.

Šeremešić, S., Marinković, D., Manojlović, M., Jovović, Z., Ćirić, V., Vasin, J., Vojnov, B. (2021b). Soil organic matter pools and aggregate fractions in organic and conventional winter wheat cropping in Vojvodina province of Serbia. Agriculture and Forestry, 67 (3): 177-189.

Šeremešić, S., Vojnov, B., Manojlović, M., Milošev, D., Ugrenović, V., Filipović, V., Babec, B. (2017). Organska poljoprivreda u službi biodiverziteta i zdravlja. Letopis naučnih radova / annals of agronomy 41(2), 51-60.

Seyed-Mohammadi, J., Esmaeelnejad, L., Ramezanpour, H. (2016). Determination of a suitable model for prediction of soil cation exchange capacity. Modeling Earth Systems and Environment 2 (156):1.

Shirazi, M. A., Boersma, L. (1984). A unifying quantitative analysis of soil texture1. Soil Science Society of America Journal J.48:142–47.

Silva, L. S., Camargo, F. A. O., Cereta, C. A. (2012). Composição da fase sólida orgânica do solo. In: Meurer EJ, editor. Fundamentos de química do solo. 5. ed. Porto Alegre: Gênesis; 61-86.

Soares, M. R., Alleoni, L. R. F. (2008). Contribution of soil organic carbon to the ion exchange capacity of tropical soils. J Sustain Agr. 2008;32:439-62.

Souza, I. F., Archanjo, B. S., Hurtarte, L. C. C., Oliveros, M. E., Gouvea, C. P., Lidizio, L. R., Achete, C. A., Schaefer, C. E. R., Silva, I. R. (2020). Al-/Fe-(hydr)oxides-organic carbon associations in Costa et al. Contribution of the chemical and mineralogical properties of sandy-loam... Rev Bras Cienc Solo 2020;44:e0200019 18 Oxisols- from ecosystems to submicron scales. Catena. 2017;154:63-72.

Soyergin, S., Moltay, I., Genç, Ç., Fidan, A. E., & Sutçu, A. R. (2002). Nutrient status of

Sposito, G. (2008). The chemistry of soils. 2nd ed. New York: Oxford University Press.

Sumner, M. E., Miller, M. P. (1996). Cation exchange capacity and exchange coefficients. In: Sparks DL, Page AL, Helmke PA, editors. Methods of soil analysis. Chemical methods. Part 3. Madison: Soil Science Society of America, 1201-29.

Sutton, R., Sposito, G., Diallo, M. S., Schulten, H. R. (2005). Molecular simulation of a model of dissolved organic matter. Environ. Toxicol. Chem. 24, 1902–1911 tablelook-up scheme and artificial neural network approach. Modern Applied Science 5(1):153–65.

Tanimu, J., Lyocks, S. W. J. (2013). Assessment of Manure Management Practices and Nitrogen Levels on Soil Phosphorus in an Alfisol. Journal of Agriculture and Sustainability, 4(2), 177–190.

Tessier, D., Biggore, F., Bruand, H. (1999). La capacité d’échange: Outil de prévision des propriétés physiques des sols. Compte Rendu d’Académie des Sciences Paris, 85(2), 37-46.

Tomašić, M., Zgorelec, Ž., Jurišić, A., Kisić, I. (2013). Cation exchange capacity of dominant soil types in the Republic of Croatia. Journal of Central European Agriculture. 14. 84-98.

Topalović, A., Knežević, M., Lekić, D. (2022). Assessment of soil fertility and leaf nutrients in olive orchards. Agriculture and Forestry, 68 (4): 31-40.

Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek P. M., Hendricks, D. M. (1997). Mineral control of soil organic carbon storage and turnover. Nature 389:170.

Totsche, K. U., Rennert, T., Gerzabek, M. H., Kögel-Knabner, I., Smalla, K., Spiteller M., Vogel, H. J. (2010). Biogeochemical interfaces in soil: The interdisciplinary challenge for soil science. Journal of Plant Nutrition and Soil Science, 173,88–99.

Tsadilas, C. D., Karaivazoglou, N. A., Tsotsolis, N. C., Stamatiadis, S., Samaras, V. (2005). Cadmium uptake by tobacco as affected by liming, N form, and year of cultivation, Environ. Pollut., 134, 239–246.

Van der Voort, T. S., Hagedorn, F., McIntyre, C., Zell, C., Walthert, L., Schleppi, P. (2016). Variability in 14C contents of soil organic matter at the plot and regional scale across climatic and geologic gradients. Biogeosci. Discuss,1–28.

Vogel, C., Mueller, C. W., Höschen, C., Buegger, F., Heister, K., Schulz, S. (2014). Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nat. Commun. 5(1), 2947.

Vojnov, B., Manojlović, M., Latković, D., Milošev, D., Dolijanović, Ž., Simić, M., Babec, B. & Šeremešić, S. (2020b). Grain yield, yield components and protein content of organic spelt wheat (Triticum spelta L.) grown in different agroecological conditions of northern Serbia. Ratarstvo i povrtarstvo, 57(1): 1-7.

Vojnov, B., Šeremešić, S., Ćupina, B., Crnobarac, J., Krstić, Đ., Vujić, S., & Živanov, M. (2019). Uticaj jarih useva gajenih naкon ozimih međuuseva na stanje sabijenosti zemljišta. Zemljište i biljka, 68(2), 72-80.

Vojnov, B., Šeremešić, S., Ćupina, B., Krstić, Đ., Vujić, S., Živanov, M., & Pavlović, S. (2020a). Hot water extractable organic carbon of chernozem under the system of cover crop incorporation and subsequent sowing of spring crops. Zemljište i biljka, 69(2), 82-94.

Weil, R., Brady, N. C. (2016). Nature and Properties of Soils, the: Pearson New International Edition. London: Pearson Higher Ed.

Wieder, W. R., Grandy, A. S., Kallenbach, C. M., Taylor, P. G., Bonan, G. B. (2015). Representing life in the Earth system with soil microbial functional traits in the MIMICS model. Geosocial. Model Dev. 8, 1789–1808.

Zitouni, H., Kabiri, G., Hanine, H., Charafi, J., Hamdani, A., Houmanat, K., Zerhoune, M. (2023): Assessment of genetic diversity of wild strawberry tree (Arbutus unedo l.) genotypes from Morocco using ISSR markers. Agriculture and Forestry, 69 (4): 135-155. doi:10.17707/AgricultForest.69.4.09

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ASSESSMENT OF GENETIC DIVERSITY OF WILD STRAWBERRY TREE (*ARBUTUS UNEDO* L.) GENOTYPES FROM MOROCCO USING ISSR MARKERS

SUMMARY

The aim of this study was to evaluate the genetic diversity among 36 wild strawberry tree (*Arbutus unedo* L.) genotypes belonging to different altitude and geographical origins, using 16 ISSR (Inter Simple Sequence Repeats) markers. Results revealed that ISSR primers produced a total of 344 bands, of which 94.1% were polymorphic, indicating a high level of genetic variation among the studied populations. The mean values of polymorphism information content (PIC), efective multiplex ratio (EMR), resolving power (Rp) and marker index (MI) were 0.34, 19.13, 23.46 and 6.45, respectively. The total gene diversity (Ht), gene diversity within populations (Hs) and total genetic differentiation coefficient among the populations (Gst) values were 0.33, 0.15 and 0.50, respectively. The genetic distance between all pairwise combinations of the genotypes ranged from 0.113 to 0.512. The cluster analysis revealed three main groups and which are subdivided into eight subgroups and a two independant branches. Therefore, the grouping of strawberry tree genotypes was independent of their geographical origins and altitude with exception for the genotypes belonging to Bin El-ouidane and Laanoucer populations. The results showed that ISSR markers are suitable tools for the evaluation of genetic diversity among strawberry tree genotypes. The data of this work will be useful for strawberry tree breeding programs and conservation strategies.

Keywords: *Arbutus unedo* L., strawberry tree, genetic diversity, ISSR markers, polymorphism, Morocco

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INTRODUCTION

Strawberry tree (*Arbutus unedo* L.), is an evergreen shrub or small tree belonging to the *Ericaceae* family and the genus *Arbutus*. It is native to Mediterranean countries such as Algeria, Morocco, Tunisia, Turkey, Syria, Greece, Croatia, France, Portugal and Spain (Serçe *et al*. 2010, Ruiz-Rodriguez *et al.* 2011). Furthermore, the plant is found in western, central and southern Europe, northeastern Africa, the Canary Islands and western Asia. It can also be found distributed in countries in North America, and on the Atlantic coast such as Ireland and Macaronesia (Canary Islands) (Celikel *et al.* 2008, Ruiz-Rodriguez *et al.* 2011, Abbas, 2015). Strawberry tree prefers siliceous or decarbonated substrata and can grow on alkaline and relatively acidic soils (pH 5-7.2) (Torres *et al*. 2002, Godinho-Ferreira *et al.* 2005, Celikel *et al.* 2008, Gomes, 2011).The species is diploid (2n=26), reproduces sexually via seeds and is capable of vegetative spreading through root suckers. It is believed as a long-distance dispersal species, and seeds are dispersed by frugivores mainly birds and mammals (Debussche and Isenmann, 1989, Aparicio *et al.* 2008). The autogamy is the most frequent mode of pollination, but the anemogamy and entomogamy may little occur (Hagerup, 1957). The plant has a huge ecological importance since it prevents erosion of the soils and has also the capacity to regenerate itself rapidly after fires, surviving quite well in poor soils (Gomes and Canhoto, 2009, Takrouni *et al.* 2012). It is widely used in traditional medicine, such as antiseptics, diuretics, and laxatives as well as for its uses in the treatment of hypertension, atherosclerosis, thrombosis (Ziyyat *et al.* 2002, Mekhfi *et al.* 2006) and diabetes (Bnouham *et al.* 2007). The strawberry tree fruit is suitable for the production of alcoholic beverages, jams, jellies, and marmalades (Pallauf *et al.* 2008).

The analysis of genetic variability within and among populations over the geographical range of the species, based on molecular markers, can minimize future risk of genetic erosion, establish forms of rational economic exploitation, and assist in the development of pertinent conservation and genetic improvement strategies (Reis, 1996). Morphological and biochemical markers have been widely exploited in breeding studies and in the investigations into diversity of species and the relationship between genotypes, and their wild parent (Vidrih *et al.* 2013). Conventional plant breeding depends on phenotypic selection in the first step (Ercisli *et al.* 2012). However, the morphological and biochemical traits have some limitation, they are strongly influenced by environmental factors and they varied with plant developmental stage (Kercher and Sytsma, 2000, Ouinsavi and Sokpon, 2010). In recent years, various types of molecular markers that are based on DNA have been developed to study genetic diversity and genotype identification of plant (Sorkheh *et al.* 2009, Zaefizadeh and Goliev, 2009). Among these genetic markers, RAPD (Randomly Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), SNPs (Single Nucleotide Polymorphic) and ISSR (Inter Simple Sequence Repeats) techniques, are commonly used for genetic studies of strawberry tree in Tunisia and Portugal (Takrouni and

Boussaid, 2010; Rodriga de Sà *et al*. 2011; Gomes *et al.* 2012; Lopes *et al*. 2012; Fazenda and Miguel, 2013; Ribeiro *et al*. 2017; Fazenda *et al*. 2019). The low reproducibility of RAPD, and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism are some limitations of these primers (Reddy *et al.* 2000, Gupta *et al.* 2010). ISSR's are best to overcome these limitations (Reddy *et al.* 2002). The ISSR markers are simple, fast and highly reproducible. They also require small amounts of DNA and do not require any prior sequence information of amplified locus (Zietkiewics *et al*. 1994, Pradeep *et al.* 2002).

Although there are works published about strawberry tree fruits in Morocco regarding biochemical composition (Zitouni *et al*. 2020, Zitouni *et al.* 2022), as well as morphological variability (Zitouni *et al.* 2021), the genetic diversity within the species is still largely unknown. Thus, the objective of this study was to evaluate a genetic diversity of strawberry tree genotypes belonging to different bioclimates and geographical origins, using ISSR molecular markers. Herein, we intend to present a complete database regarding genetic diversity of Moroccan strawberry tree in order to provide useful information for future conservation of these genetic resources and breeding programs.

MATERIAL AND METHODS

Plant material

Thirty-six genotypes were selected from natural populations of Strawberry tree (*Arbutus unedo* L.) representing the natural range of the species in Morocco.

Table 1. The geographical characteristics of the studied strawberry tree genotypes

Localities	Sample Code	Geographic Zone	Longitude (W)	Latitude (N)	Altitude (m)
Bin El Ouidane	BNO	Middle Atlas	6°30'	32°05'	1313
Tamscart	TAM	Middle Atlas	6°16'	32°16'	1520
Ksiba	KSB	Middle Atlas	6°11'	32°31'	1338
Ouaouizerth	OUA	Middle Atlas	6°21'	32°09'	1050
Tahnaout	TAH	High Atlas	7°55’	31°18’	1200
Khénifra	KHN	Middle Atlas	5’27’	32°53’	1613
Laanoucer	LAN	Middle Atlas	4°54’	33°42’	1700
Oulmès	OUL	Central Plateau	6°02’	33°33’	935
Chefchaoun	CHF	Western Rif	5°17’	35°07’	534
Ouazzane	OUZ	North West	5°32’	34°55’	202
Moulay Driss Zerhoune	MDZ	Middle Atlas	5°30’	34°02’	820
Bab Marzouka	BMR	Rif- Middle Atlas	4°08’	34°10’	801

The main geographical characteristics of the populations are reported in Table 1 and Figure 1.

Young leaves were collected and stored at -80°C, then the samples were freeze-dried in a WPA Bio-wave S2100 freeze-dryer to be used for DNA extraction.

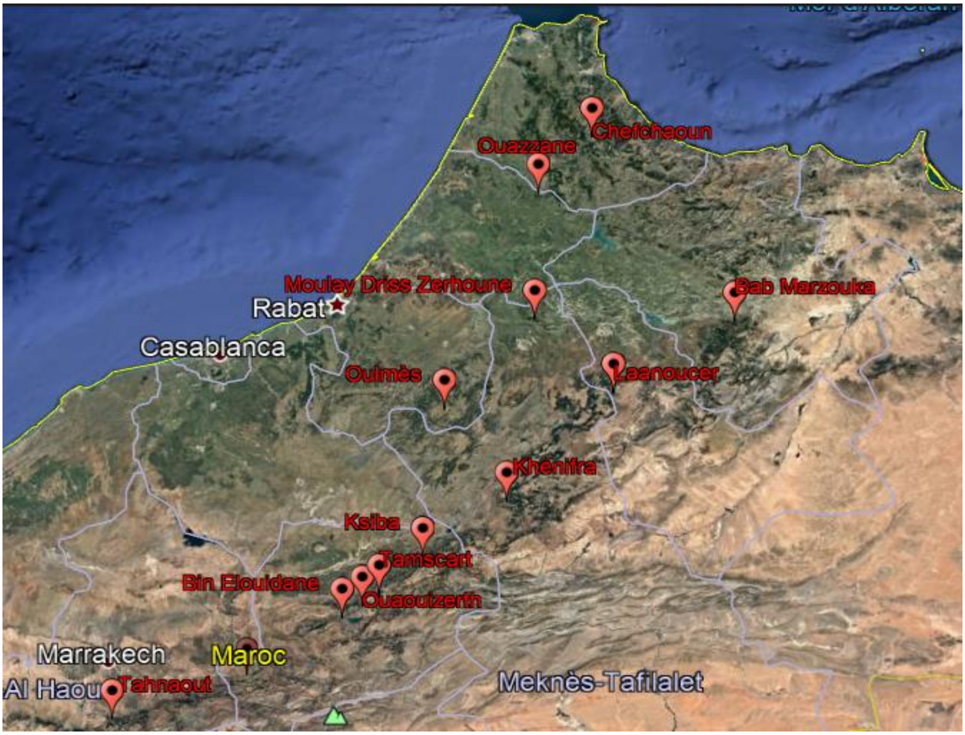

Figure 1. Geographical Origin of the studied strawberry tree (*Arbutus unedo* L.) populations

DNA extraction and quantification

Genomic DNA was extracted from young leaves using the Cetyl Trimethyl Ammonium Bromide (CTAB) method described by Saghai-Maroof *et al.* (1984). The quantity and quality of the DNA obtained was assessed spectrophotometrically by a Nanodrop (BioDrop µLite+, 2019) at 230, 260 and 280 nm.

ISSR primer screening

A total of 20 ISSR primers was tested and 16 primers were selected (Table 2). Gradient PCR was used to adjust the annealing temperature of each primer.

Table 2. The 16 ISSR primers used in this study and their annealing temperature

Primers	Sequence (5'-3')	H.T (°C)	BS (bp)
F2	[CA] 6GC	54.8	141 - 4392
F8	[AG] 8CC	50.7	188 - 3587
F10	[CA] 8AG	42	208 - 4676
F11	[CA] 8AC	54.8	162 - 5319
F12	[GA] 8CC	45.4	594 - 14000
IMA 834-1	[AG] 8YT	53.5	134 - 2441
IMA 834-2	[AG] 8YT	45.4	362 - 13362
IMA 834-Z	[AG] 8YT	50.7	281 - 8985
IMA 9-Z	[GA] 8CG	54.8	156 - 3412
IMA 12-1	[CA] 8TC	50.7	177 - 3632
UBC 808-2	[AG] 8C	54.8	145 - 3371
UBC 807	[AG] 8T	50	402 - 7143
UBC 817	[CA] 8A	50	470 - 11244
UBC 818	[CA] 8G	42	216 - 3153
ISSR 1	[AG] 8CA	45.4	457 - 11267
ISSF 1	[AG] 8TA	45.4	654 - 8094

H.T: Hybridization temperature, BS: band size

Statistical analysis

Band size was measured with Mesurim Pro V3.4 software. A binary data matrix was created based on the presence of bands (1) or their absence (0) in each genotype. The polymorphism information content (PIC), linked to the genetic diversity for each primer, was evaluated according to the formula of De Riek *et al.* (2001), PIC=2i (1-fi), where f is the band frequency in the data set. The Effective multiple ratio (EMR) was obtained according to Powell *et al.* (1996) as follow: EMR=np (np/n), where np is the number of polymorphic loci and n is the total number of loci. The Marker index (MI) was estimated according to Chesnokov and Artemyeva, (2015) as the product of PIC value and EMR value (MI=PIC×EMR). The Marker index (MI) is a statistical parameter used to estimate the total utility of the maker system. The Resolving power (Rp) of each ISSR primer was calculated according to Prevost and Wilkinson, (1999), as follow: $Rp=\sum lb$, where $lb=1-[2\times(0.5-p)]$, where p is the proportion of genotypes containing the i th band. Parameters of genetic diversity were calculated: numbers of alleles (Na), effective number of alleles (Ne), the diversity within the populations (Hs), total gene diversity (Ht), coefficient of gene differentiation (GST) using POPGENE version 1.32 software (1999). Genetic distances were calculated using Simple Coefficient Matching (SCM) by Clustering Calculator software program established by Brzustowski, (2002). A histogram of pairwise comparisons between all genotypes according to the number of markers that differentiate them was established. Then, a dendrogram was constructed on the basis of the similarity matrix data using an Unweighted Pair Group Mean with Arithmetic Average (UPGMA) method to better understand the patterns of variability among the genotypes.

RESULTS AND DISCUSSION

ISSR polymorphism

The 16 ISSR primers amplifed produced a total of 344 bands, which 324 were polymorphic. The band size ranged from 134 to 14000 base pairs (Table 2). The number of amplified fragments ranging from 14 bands (IMA 834-1) to 28 bands (F2) with an average of 21.5. The number of polymorphic bands per primer varied from 14 (ISSF1, IMA 834-1) to 27 (F2), with a mean of 20.25. Moreover, the percentage of polymorphism ranged from 86.9% for ISSR1 and F8 primers to 100% for F12, IMA 834-2, IMA 834-1, F11, F10 and UBC 818, with an average of 94.1% (Table 3). The high percentage of polymorphic bands per primer revealed the high level of polymorphism showed by the studied primers among the strawberry tree genotypes.

The Polymorphism Information Content (PIC) measures the ability of the markers to detect polymorphisms, the PIC values ranging from 0.28 for primer ISSF1 to 0.38 for primers F2, F11 and UBC 817 with a mean value of 0.34 (Table 3).

Regarding the effective multiplex ratio (EMR), the highest value of EMR (26.04) and the lowest (12.25) were observed for primers IMA 12-1 and F8, respectively, with an average of 19.13. The marker index (MI) used to estimate the overall utility of each marker system (Sorkheh *et al.* 2007). The values of MI ranged from 3.80 in the primer F8 to 9.56 in the primer F2 with a mean of 6.45. The F2 primer seems to be the most appropriate primer it had the highest value of PIC and MI and consequently is the most informative. Furthermore, the resolving power (Rp) which indicates to the efficiency of the ISSR primers used in discriminating strawberry tree genotypes, the values varied from 14.61 for the IMA 834-1 primer to 34 for F12 with an average value of 23.46 (Table 3). In general, the use of ISSR markers was found to be very efficient and yielded a high polymorphism rate.

The number of bands obtained in the current study with an average value of 21.5 bands were higher than those recorded by Lopes *et al.* (2012) in 46 Portuguese strawberry tree genotypes. They found a total of 45 and 56 bands using 5 ISSR and 6 RAPD primers with an average value of 9 and 9.3 bands, respectively. Gomes *et al.* (2012) tested 20 RAPD primers across 27 Portuguese strawberry tree genotypes and they found 124 bands, that ranged from 1 to 13 with a mean value of 6.2 bands. In another study, Takrouni and Boussaid, (2010) assessed the genetic diversity of Tunisian strawberry tree using 9 RAPD markers. They reported a total of 88 bands, out of which 65 were polymorphic, that varied between 8 and 13 bands per primer.

Table 3. Characteristics and amplification results of 16 ISSR primers in the studied genotypes

Primer	TNB	NPB	NMB	PPB (%)	PIC	EMR	MI	Rp
F2	28	27	0	96.43	0.38	25	9.56	23.22
F8	23	20	2	86.96	0.31	12.25	3.80	24
F10	23	23	0	100	0.35	23	8.00	23.05
F11	26	26	1	100	0.38	20.05	7.62	22.55
F12	25	25	3	100	0.35	17.39	6.02	34
IMA 834-1	14	14	2	100	0.37	13.24	4.88	14.61
IMA 834-2	23	23	2	100	0.37	16.2	5.99	24
IMA 834-Z	22	21	0	95.45	0.30	14	4.15	22.16
IMA 9-Z	21	19	1	90.48	0.33	21.04	7.02	22
IMA 12-1	23	22	1	95.65	0.31	26.04	7.99	23.11
UBC 808-2	25	22	0	88	0.31	26	8.18	28.22
UBC 807	17	15	3	88.23	0.36	17.39	6.18	21.94
UBC 817	20	18	0	90	0.38	23	8.66	22
UBC 818	15	15	2	100	0.33	17.19	5.67	18.22
ISSR 1	23	20	3	86.96	0.30	19.36	5.82	29.61
ISSF 1	16	14	0	87.5	0.28	15	4.19	22.66
Average	21.5	20.25	1.25	94.10	0.34	19.13	6.45	23.46

TNB: Total number of bands, NPB: Number of polymorphic bands, NMB: Number of monomorphic bands, PPB: Percentage of polymorphic bands, EMR: Effective multiple ratio, MI: Marker index, PIC: polymorphic information content, Rp: Resolving power.

Comparing our results to previous studies carried out in other species belonging to *Ericaceae* family, the value of total markers found by Carvalho *et al.* (2018) was lower than the one we obtained in the current study. The 10 ISSR primers used by these authors to analyzed 16 blueberry (*Vaccinium corymbosum*) cultivars and three wild populations of *Vaccinium myrtillus* produced a total of 122 markers among the genotypes. Generally, the high total number of bands found in our research could be explained by the relatively larger number of primers used and by the types of genetic material used.

The results concerning the percentage of polymorphism detected in our study (94.10%) were higher than those reported in Portuguese strawberry tree by Lopes *et al.* (2012) using ISSR (86.7%) and RAPD (83.9%) markers and by Gomes *et al.* (2012) based on RAPD (57.3%) and SSR markers (80%) and by Rodriga de Sà (2010) using RAPD (83.9%) and ISSR (86.7%) markers, and in Tunisian strawberry tree by Takrouni and Boussaid (2010) using RAPD (65%) and Takrouni *et al.* (2012) using isozymes (63.33%). In other reports, the mean value of polymorphism detected by Carvalho *et al*. (2018) in blueberry (*Vaccinium corymbosum*) cultivars was lower (83.20%) than the value obtained in strawberry tree genotypes.

According to Roldan-Ruiz *et al.* (2000), the closer the PIC value to 0.5, the higher the polymorphism index of the primer in question. In our study, the primers F2, F11 and UBC 817 had the highest values of PIC, indicating that these primers are informative and presented a high performance in the genetic exploration of this species. Gomes *et al.* (2012) obtained much higher average PIC value of 0.71 in Portuguese strawberry tree using 11 SSR markers. Comparing our results to previous studies carried out in *Vaccinium* species, the PIC average value (0.30) reported by Carvalho *et al.* (2018) in *Vaccinium corymbosum* cultivars was lower than the value obtained in our study. For *Vaccinium myrtillus* populations, the mean value of PIC obtained by the same researchers was much lower (0.05) than our results. In plum (*Prunus salicina* L) genotypes, Hamdani *et al*. (2022) reported similar average value of PIC (0.34) using 20 ISSR primers.

Primers with high Rp values were generally more effective in distinguishing between genotypes and showed higher number of polymorphic bands (Prevost and Wilkinson, 1999, Debnath, 2007). In our study, the primer F12 showed to be the most effective (Rp=34). Comparing the results obtained in this work to previous studies carried out in *Vaccinium species*, the value of Rp found in strawberry tree was higher than those reported by Debnath, (2007), Debnath, (2009) and Carvalho *et al*. (2018). The Rp values recorded by Debnath, (2007) varied between 2.1 and 9.4 in 43 lingonberry (*Vaccinium vitis-idaea* L.) clones collected from four Canadian provinces using 15 ISSR primers. In another study, Debnath, (2009) reported values of Rp ranged from 1.9 to 8.1 in 43 wild lowbush blueberry (*Vaccinium angustifolium*) clones by using ISSR markers. In Portugal, Carvalho *et al.* (2018) analyzed *Vaccinium corymbosum* and *Vaccinium myrtillus* cultivars using 10 ISSR primers and the Rp values were 11.40 and

14.50, respectively. The results concerning MI (6.45) were lower than those found by Carvalho *et al.* (2018). They found an average value of 24.60 in *Vaccinium corymbosum* cultivars, whereas they reported a mean value much lower (0.50) in *Vaccinium myrtillus* using ISSR markers. The results obtained clearly demonstrate that ISSR markers are a high efficient marker to characterize strawberry tree genotypes, as has been referred in other horticultural species.

Genetic diversity analysis

The results of genetic diversity analysis were presented in Table 4. The number of alleles observed (Na) varied from 1.87 (ISSF1, UBC 807 and UBC 817) to 2 (F12, IMA 834-2, IMA 834-Z, IMA 834-1, F11 and UBC 818) with an average of 1.94. The mean effective number of alleles (Ne) was 1.55, the values ranged from 1.45 for F8 to 1.68 for F12. Moreover, the Shannon information index (I) values ranged from 0.43 for F8 to 0.56 for F12 and F11 with a mean of 0.49. The total gene diversity (Ht) and the gene diversity within populations (Hs) ranged respectively, from 0.28 (F8) to 0.38 (F12, F11) and from 0.11 (F8) to 0.20 (F11), with an average of 0.33 and 0.15. The coefficient of genetic differentiation (Gst) among the populations was 0.50 and the mean value of gene flow (Nm) was 0.75 (Table 4).

Table 4. Genetic diversity analysis of the studied strawberry tree genotypes

ISSR Loci	Simple size	Na	Ne	I	Ht	Hs	Gst	Nm
F12	36	2	1.68	0.56	0.38	0.19	0.51	0.62
ISSF1	36	1.87	1.48	0.46	0.30	0.13	0.54	0.54
IMA 834-2	36	2	1.58	0.52	0.35	0.17	0.53	0.65
IMA 834-Z	36	2	1.51	0.46	0.30	0.17	0.39	1.5
ISSR1	36	1.92	1.52	0.48	0.32	0.15	0.50	0.68
UBC 807	36	1.87	1.55	0.48	0.32	0.13	0.56	0.55
UBC 817	36	1.87	1.48	0.44	0.29	0.14	0.50	0.63
IMA 834-1	36	2	1.56	0.51	0.34	0.18	0.43	0.96
IMA 12-1	36	1.96	1.56	0.49	0.33	0.13	0.56	0.55
F2	36	1.96	1.51	0.47	0.31	0.16	0.42	1.11
F11	36	2	1.66	0.56	0.38	0.20	0.43	1.01
F8	36	1.88	1.45	0.43	0.28	0.11	0.52	0.83
F10	36	1.96	1.53	0.49	0.32	0.15	0.51	0.75
IMA 9Z	36	1.91	1.57	0.49	0.33	0.15	0.51	0.65
UBC 808-2	36	1.88	1.56	0.48	0.32	0.12	0.60	0.49
UBC 818	36	2	1.59	0.53	0.35	0.16	0.53	0.51
Mean		1.94	1.55	0.49	0.33	0.15	0.50	0.75

Na: Observed number of alleles; Ne: Effective number of alleles; I: Shannon's Information index; Ht: Total genetic diversity; Hs: Genetic diversity within group; Gst: Genetic differentiation among group; Nm: Gene flow

The number of alleles observed (Na) with an average of (1.94) was higher than that obtained in Portuguese strawberry tree by Lopes *et al.* (2012) (Na=1.66) using ISSR markers, but lower than that showed by Gomes *et al.* (2012) using SSR markers (Na=11.6) for Portuguese strawberry tree. Our results regarding the Shannon's information index (I=0.49) given by the primers tested was higher than that found in Portuguese strawberry tree by Lopes *et al.* (2012) using ISSR markers. They found an average value of I=0.39 that vary between 0.29 and 0.52.

The high value of (Ht=0.33) suggests the presence of a high level of polymorphism. This value was similar to that reported by Rodriga de Sà, (2010) (Ht=0.33) in the Portuguese strawberry tree using RAPD and ISSR markers and by Kabiri *et al.* (2022) (Ht=0.33) in Moroccan *Juglans regia* using ISSR markers. However, it was higher than that obtained by Lopes *et al*. (2012) (Ht=0.30) in Portuguese strawberry tree using ISSR markers. Indeed, the recorded polymorphism is confirmed by the Shannon index value (0.49). The high genetic diversity obtained in Moroccan strawberry tree was generally in agreement with general trend for all plant species (Ht=0.30 from 584 entries), long-lived woody perennial species (Ht=0.28 from 195 entries) (Hamrik *et al*. 1992). The high diversity of Moroccan strawberry tree genotypes could be explained by seed and pollen migration between populations (Mesléard and Lepart, 1991). It could be attributed also to life history traits of this species.

Nevertheless, our results showed low levels of variation within strawberry tree populations (Hs=0.15). The Hs value found in this study was relatively lower than the results recorded by other authors. In Portuguese strawberry tree, Lopes *et al.* (2012) recorded low to moderate levels of variation within populations (Hs=0.23) using ISSR markers. In Tunisia, Takrouni and Boussaid, (2010) reported also, a low level of genetic variation within strawberry tree populations (Hs=0.21) using RAPD markers.

This low level of variation could be the resulted of inbreeding, dictated by the selfing mating system and by the small population size. According to Barrett and Kohn, (1991), small population sizes can cause both genetic drift and inbreeding leading to loss of genetic variation within populations. Moreover, the failure of strawberry tree seeds to germinate, due to specific requirements for seed germination (Mereti *et al*. 2003) would result in the population decline and in the decrease of genetic diversity. In other reports, has been documented that selfers plant populations have, in general, low level of genetic variation (Rossetto *et al.* 1995, Hamrick and Godt, 1996, Godt *et al*. 2001).

The mean value of Gst among the populations was large (Gst=0.505), indicating that 50.5% of total genetic variability was distributed among the populations, while 49.5 % of the total genetic diversity was within populations. Depending on the obtained Gst value, Moroccan strawberry tree genotypes were largely differentiated. Our results were higher than that reported by Lopes *et al.* (2012) (Gst=0.22) and by Rodriga de Sà, (2010) (Gst=0.26) in Portuguese strawberry tree using ISSR markers, and by Ribeiro *et al*. (2017) (Gst=0.29) using SSR markers for Portuguese strawberry tree. In addition, our results were higher than that found by Takrouni and Boussaid, (2010) (Gst=0.31) in Tunisian strawberry tree using RAPD markers. According to Slatin, (1987), a value of Gst>0.25 is generally regarded as the threshold quantity beyond which significant population differentiation occurs.

The high level of differentiation between populations was in agreement with the restricted gene flow (0.75) that could be due to presence of geographical barriers. According to Wright, (1978), the Nm was divided into three grades: high (≥1), medium (0.25-0.99) and low (0-0.249) (Govindaraju, 1988), and when Nm>1, there was certain gene flow between populations. Previous work showed that if gene flow Nm<1, genetic drift was the main factor affecting the genetic structure of the populations, while if Nm>1, gene flow was sufficient to counteract the effect of genetic drift, and also to prevent the occurrence of genetic differentiation between populations (Levin, 1984).

Genetic distance

The genetic distance between the different strawberry tree genotypes was calculated based on the 344 bands obtained. The genetic distance varied from 0.113 to 0.520 (Table 5 and 6), with an average of 0.343.

Table 5. Genetic distances between the studied strawberry tree genotypes

	CHF1	CHF2	CHF3	OUZ1	OUZ2	OUZ3	MDZ1	MDZ2	MDZ3	LAN1	LAN2	LAN3	BMR1	BMR2	BMR3	OUL1	OUL2	OUL3
CHF2	0,238																	
CHF3	0,172	0,172																
OUZ1	0,358	0,363	0,326															
OUZ2	0,413	0,465	0,427	0,311														
OUZ3	0,491	0,497	0,488	0,244	0,259													
MDZ1	0,262	0,250	0,235	0,299	0,424	0,387												
MDZ2	0,326	0,372	0,363	0,317	0,331	0,317	0,331											
MDZ3	0,291	0,349	0,340	0,311	0,366	0,346	0,314	0,320										
LAN1	0,381	0,422	0,401	0,390	0,299	0,326	0,358	0,375	0,288									
LAN2	0,410	0,445	0,465	0,390	0,276	0,291	0,410	0,340	0,323	0,297								
LAN3	0,410	0,445	0,424	0,401	0,340	0,326	0,392	0,311	0,294	0,273	0,262							
BMR1	0,456	0,456	0,453	0,378	0,259	0,279	0,398	0,317	0,334	0,279	0,326	0,267						
BMR2	0,480	0,485	0,483	0,384	0,305	0,297	0,410	0,328	0,352	0,308	0,291	0,291	0,233					
BMR3	0,395	0,413	0,410	0,346	0,273	0,305	0,395	0,314	0,355	0,311	0,305	0,299	0,235	0,224				
OUL1	0,439	0,456	0,448	0,378	0,328	0,302	0,416	0,311	0,346	0,314	0,291	0,297	0,291	0,308	0,288			
OUL2	0,305	0,363	0,302	0,302	0,358	0,331	0,323	0,340	0,270	0,302	0,343	0,326	0,326	0,390	0,340	0,291		
OUL3	0,282	0,334	0,273	0,302	0,311	0,355	0,282	0,311	0,276	0,326	0,302	0,297	0,326	0,331	0,305	0,331	0,203	
KHN1	0,291	0,349	0,328	0,311	0,326	0,358	0,314	0,256	0,355	0,352	0,363	0,340	0,369	0,352	0,343	0,346	0,334	0,288
KHN2	0,363	0,416	0,390	0,302	0,311	0,279	0,352	0,265	0,305	0,308	0,326	0,308	0,250	0,291	0,241	0,285	0,291	0,291
KHN3	0,285	0,291	0,265	0,311	0,355	0,416	0,256	0,395	0,326	0,375	0,387	0,404	0,398	0,439	0,366	0,433	0,334	0,282
TAH1	0,288	0,363	0,326	0,308	0,299	0,349	0,317	0,323	0,282	0,314	0,302	0,291	0,314	0,349	0,270	0,297	0,291	0,262
TAH2	0,366	0,395	0,404	0,328	0,302	0,340	0,372	0,326	0,384	0,346	0,305	0,328	0,323	0,311	0,331	0,294	0,328	0,305
TAH3	0,302	0,314	0,328	0,346	0,343	0,387	0,337	0,343	0,349	0,363	0,375	0,387	0,358	0,416	0,343	0,363	0,299	0,294
OUA1	0,323	0,363	0,355	0,343	0,358	0,384	0,340	0,334	0,323	0,331	0,343	0,349	0,355	0,401	0,346	0,337	0,302	0,308
OUA2	0,241	0,270	0,244	0,326	0,427	0,500	0,294	0,410	0,346	0,395	0,436	0,442	0,471	0,512	0,398	0,465	0,331	0,308
OUA3	0,253	0,317	0,256	0,273	0,404	0,436	0,270	0,375	0,317	0,395	0,407	0,401	0,442	0,471	0,369	0,424	0,308	0,291
BNO1	0,334	0,398	0,384	0,291	0,334	0,360	0,381	0,288	0,282	0,372	0,349	0,343	0,355	0,349	0,340	0,360	0,320	0,279
BNO2	0,355	0,401	0,358	0,311	0,331	0,363	0,372	0,302	0,308	0,369	0,334	0,317	0,346	0,340	0,343	0,352	0,323	0,282
BNO3	0,384	0,372	0,369	0,363	0,378	0,410	0,366	0,366	0,349	0,404	0,398	0,433	0,410	0,416	0,355	0,427	0,358	0,334
KSB1	0,267	0,331	0,294	0,340	0,384	0,427	0,302	0,326	0,256	0,369	0,358	0,346	0,346	0,369	0,314	0,346	0,270	0,247
KSB2	0,244	0,314	0,265	0,363	0,430	0,509	0,326	0,436	0,326	0,387	0,445	0,474	0,485	0,520	0,448	0,485	0,334	0,328
KSB3	0,279	0,337	0,288	0,363	0,372	0,433	0,302	0,349	0,326	0,381	0,363	0,369	0,381	0,392	0,337	0,410	0,323	0,282
TAM1	0,390	0,349	0,358	0,381	0,366	0,392	0,401	0,384	0,390	0,392	0,369	0,398	0,369	0,410	0,297	0,381	0,340	0,340
TAM2	0,430	0,413	0,416	0,427	0,413	0,427	0,442	0,360	0,459	0,468	0,387	0,445	0,410	0,416	0,366	0,422	0,375	0,369
TAM3	0,459	0,477	0,468	0,381	0,273	0,299	0,430	0,320	0,384	0,334	0,311	0,299	0,253	0,276	0,227	0,276	0,358	0,334

The genotypes were dissimilar with a number of markers ranged from 39 to 176 using a pairwise comparisons (Figure 2). The lowest genetic distance (0.113) was found between the genotypes BNO2 and BNO1 with only 39 dissimilar markers differentiated both genotypes. The genetic similarity between the genotypes can be explained by the geographic proximity as well as the common ancestor. However, the highest genetic distance (0.520) was recorded between the genotypes KSB2 and BMR2, indicating that these genotypes are genetically distinct with 176 dissimilar markers.

The variation of genetic distances (0.113-0.520) was similar to the results of Hamdani *et al.* (2022), who reported values of genetic distances ranged between 0.136 and 0.619 with a mean of 0.365 in plum (*Prunus salicina* L) genotypes using 20 ISSR primers.

Table 6. Genetic distances between the studied strawberry tree genotypes (suite)

	KHN1	KHN2	KHN3	TAH1	TAH2	TAH3	OUA1	OUA2	OUA3	BNO1	BNO2	BNO3	KSB1	KSB2	KSB3	TAM1	TAM2
KHN2	0,299																
KHN3	0,291	0,328															
TAH1	0,247	0,250	0,235														
TAH2	0,233	0,259	0,337	0,265													
TAH3	0,291	0,328	0,331	0,288	0,273												
OUA1	0,294	0,273	0,305	0,279	0,323	0,294											
OUA2	0,328	0,401	0,235	0,302	0,422	0,299	0,302										
OUA3	0,334	0,366	0,259	0,320	0,369	0,305	0,302	0,192									
BNO1	0,270	0,291	0,392	0,285	0,276	0,299	0,320	0,366	0,285								
BNO2	0,291	0,282	0,390	0,270	0,291	0,320	0,311	0,392	0,334	0,113							
BNO3	0,372	0,346	0,355	0,369	0,355	0,366	0,381	0,381	0,311	0,212	0,233						
KSB1	0,314	0,311	0,285	0,259	0,331	0,279	0,259	0,294	0,253	0,288	0,302	0,337					
KSB2	0,390	0,427	0,308	0,328	0,442	0,314	0,317	0,230	0,282	0,375	0,366	0,424	0,273				
KSB3	0,326	0,358	0,279	0,270	0,360	0,326	0,299	0,288	0,276	0,334	0,308	0,349	0,180	0,244			
TAM1	0,401	0,352	0,326	0,358	0,407	0,372	0,375	0,352	0,317	0,410	0,395	0,279	0,355	0,442	0,285		
TAM2	0,436	0,392	0,413	0,392	0,407	0,395	0,456	0,433	0,392	0,439	0,430	0,320	0,430	0,488	0,360	0,157	
TAM3	0,360	0,270	0,436	0,305	0,326	0,390	0,346	0,480	0,462	0,358	0,320	0,424	0,390	0,506	0,378	0,326	0,349

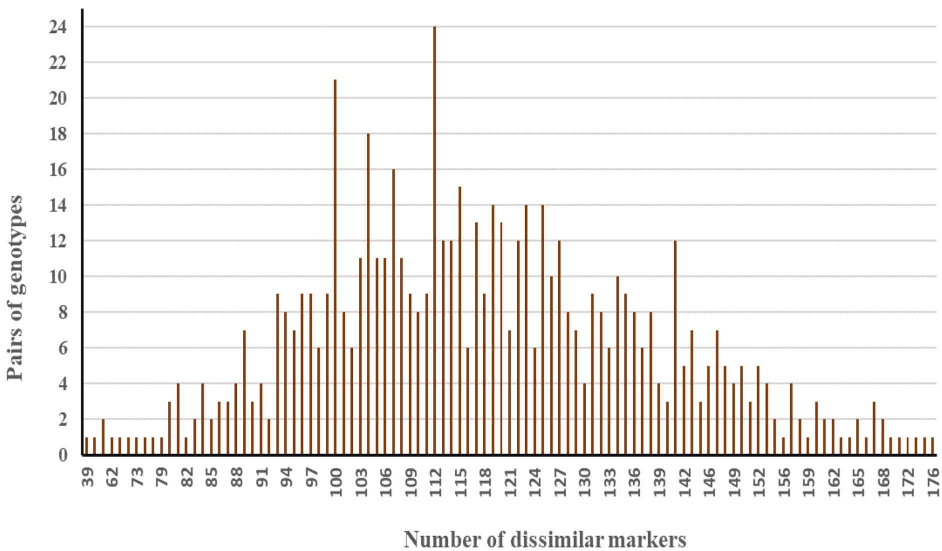

Figure 2. Frequency distribution of genetic dissimilarity for all pairwise combinations among the studied Moroccan strawberry tree genotypes

Mantel test performed in the Portuguese strawberry tree genotypes by Ribeiro et al. (2017) showed no correlation between genetic and geographic distances. Similarly, no correlation between geographic and genetic distances was found by Gomes *et al*. (2012) on Portuguese strawberry tree ($r=0.01$, $p<0.57$). However, Rodriga de Sà, (2010), showed that there was a positive correlation between geographical and genetic distances ($r=0.53$, $p<0.001$) in the Portuguese strawberry tree genotypes.

Cluster Analysis

A dendrogram, based on the genetic distance matrix, was created using the UPGMA (Unweighted Pair Group Method using Arithmetic Averages) cluster analysis (Figure 3). Cluster analysis data revealed three main groups, with two independent branches (MDZ2, OUA1). The first group divided into twenty genotypes (the largest group) subdivided into four main subgroups. The first subgroup was constituted by eleven genotypes belonging to the different altitudes and geographical zones. This subgroup contained the genotypes (CHF1, CHF3 and CHF2) from the Western Rif (very low altitude), the genotypes (MDZ1, KHN3, OUA2, OUA3, KSB2, KSB1 and KSB3) from the Middle Atlas (low and moderate altitudes) and the genotype (TAH1) from the High Atlas (moderate altitude). The second subgroup was mainly formed by the genotype (MDZ3) from the Middle Atlas and the genotypes (OUL2 and OUL3) from the Central Plateau. This group characterized by low altitudes. The third minor subgroup contained the genotype (KHN1) from the Middle Atlas and the genotypes (TAH2 and TAH3) from the High Atlas. This group presented moderate altitudes. The last subgroup included only the genotypes (BNO1, BNO2 and BNO3) from the Middle Atlas with moderate altitude. The second group included twelve genotypes subdivided into three main subgroups. The first subgroup contained only two genotypes (OUZ1 and OUZ3) from the North West (very low altitude). The second subgroup included the genotype (OUZ2) from the North West (very low altitude), the genotypes (BMR1, BMR2 and BMR3) from the Rif- Middle Atlas (low altitude), the genotype (OUL1) from the Central Plateau (low altitude) and the genotypes (TAM3 and KHN2) from the Middle Atlas (moderate altitudes). The last subgroup contained only the genotypes (LAN1, LAN2 and LAN3) from the Middle Atlas (moderate altitude). The third group included only two genotypes (TAM1 and TAM2) from the Middle Atlas (moderate altitude) (Table 7).

Both morphological and molecular dendrogram clustered the genotypes into three main groups but the genotypes did not grouped in the same cluster. In molecular dendrogram, Bin El Ouidane and Laanoucer genotypes formed distinct subgroups. However, in morphological dendrogram, these genotypes did not formed separate groups. Generally, the molecular data (ISSR loci) dit not showed positive correlation with of the morphological traits (data not shown). There are several studies on different fruit trees such as plums (Shimada *et al*., 1999) and grapevine (Vidal *et al*., 1999) reported correlation between molecular data and morphological traits. However, there is no need to be a necessarily positive correlation between morphological and molecular markers (Zhang *et al.*, 2010).

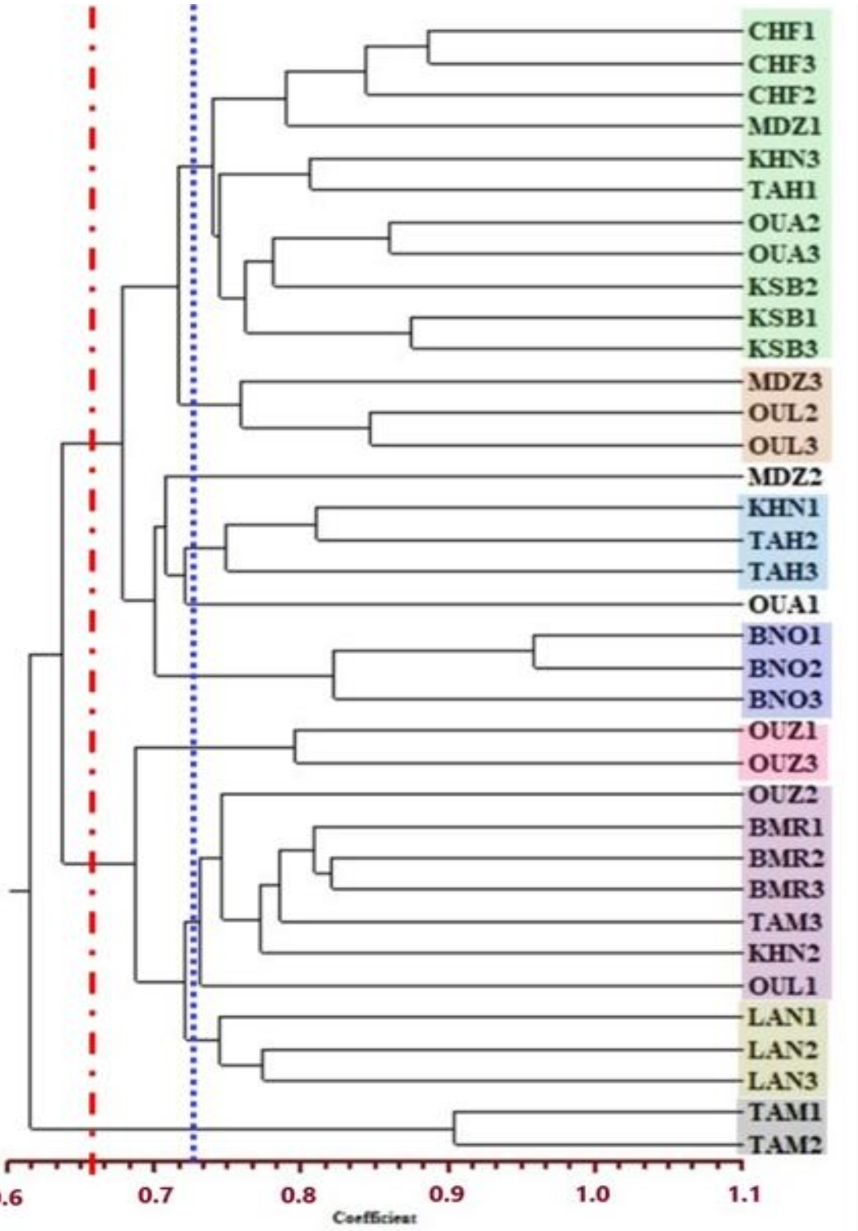

Figure 3. Dendrogram of the studied Moroccan strawberry tree genotypes generated by ISSR using UPGMA cluster analysis

Table 7. Genotypes clustering obtained from ISSR markers using UPGMA method

Groups	Subgroups	Genotypes	Total number of genotypes
Group I	Subgroup 1	CHF1, CHF3, CHF2, MDZ1, KHN3, TAH1, OUA2, OUA3, KSB2, KSB1 and KSB3.	11
	Subgroup 2	MDZ3, OUL2 and OUL3.	3
	Subgroup 3	KHN1, TAH2 and TAH3.	3
	Subgroup 4	BNO1, BNO2 and BNO3.	3
Group II	Subgroup 1	OUZ1 and OUZ3.	2
	Subgroup 2	OUZ2, BMR1, BMR2, BMR3, TAM3, KHN2 and OUL1.	7
	Subgroup 3	LAN1, LAN2 and LAN3.	3
Group III		TAM1 and TAM2	2
Branches		MDZ2 and OUA1.	2

Nevertheless, the genetic relationship observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to morphological traits (Métais *et al*., 2000).

The dendrogram obtained based on UPGMA analysis with ISSR data, did not cluster together the genotypes according to their population affinity. The finding revealed that the grouping of strawberry tree genotypes was independent of their geographic origin with exception for the genotypes belonging to Bin El Ouidane and Laanoucer populations. These genotypes formed distinct subgroups. The results showed also that the genotypes KHN1, KHN2 and KHN3 appeared dispersed throughout the groups I and II.

Lopes *et al*. (2012) clustered forty-six Portuguese strawberry tree genotypes in two main groups, based on ISSR markers. They found that the genotypes from the same population did not cluster together, with the exception for some genotypes. In another study, Ribeiro *et al.* (2017) identifed four groups, based on SSR markers, in Portuguese strawberry tree genotypes belonging to different ecological conditions. Twenty seven strawberry tree genotypes were screened by Gomes *et al.* (2012) with 20 RAPD and 11 SSR markers. They reported that clustering of Portuguese strawberry tree was not correlated to their geographical origin. Also, Takrouni and Boussaid, (2010) assessed the genetic diversity of nine Tunisian strawberry tree populations using RAPD markers. They showed the lack of correlation between population groupings and geographic origin. However, Rodriga de Sà, (2010), showed that the clustering of Portuguese strawberry tree populations resulting from the UPGMA dendrogram is in agreement with their distant geographical positions. The grouping of genotypes belonging to several geographic origin could be attributed to environmental similarities (temperature and rainfall) (Lopes *et al.* 2012).

In general, the differentiation among strawberry tree populations could result from many factors such as the length of the species vegetative period, recent fragmentation of a large initial population, and long-distance seed dispersal facilitated by frugivores, primarily birds (Debussche and Isenmann, 1989, Aparicio *et al.* 2008). Long-distance seed dispersal influences colonization of new habitats, the species' ability to migrate, and the spatial genetic structuring of populations (Aparicio *et al.* 2008). As with many autogamous species, the differentiation among Moroccan strawberry tree populations was not related to geographic distance (Hamrick and Godt, 1990).

CONCLUSIONS

The finding of this study revealed that ISSR markers are highly informative and effective in the detection of polymorphism in strawberry tree genotypes. These markers are suitable tools for the evaluation of genetic diversity among strawberry tree genotypes. The cluster analysis revealed three main groups and two independent branches. The grouping of genotypes was not related with their altitude and geographical origins with exception for the genotypes belonging to Bin El-ouidane and Laanoucer populations. The present work provides important data that could be exploited for future conservation of strawberry tree and various breeding programs. The results obtained with ISSR markers will be confronted with the morphological and biochemical evaluation of the genotypes investigated in the present work.

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REFERENCES

Abbas, A. (2015). Caractérisation physico-chimique du fruit de l'arbousier (*Arbutus unedo* L.) du nord Algérienne et de la datte (Mech-Degla), Université M'Hamed Bougara-Boumerdes, Boumerdes, Algérie.

Aparicio, A. Albaladejo, R.G. Olalla-Tarraga, M.A. Carrillo, LF. & Rodriguez, M.A. (2008). Dispersal potentials determine responses of woody plant species richness to environmental factors in fragmented Mediterranean landscapes. For Ecol Manage, 255: 2894-2906.

Barrett, S.C.H. & Kohn, J.R. (1991). Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. In: Falk, D.A., Holsinger, K.A., Eds., Genetics and Conservation of Rare Plants, Oxford University Press, New York, p 3–30.

Bnouham, M. Merhfour, F.Z. Legssyer, A. Mekhfi, H. Maallem, S. & Ziyyat, A. (2007). Antihyperglycemic activity of *Arbutus unedo, Ammoides pusilla* and *Thymelaea hirsute*. Pharmazie, 62: 630–932.

Brzustowski, J. (2002). Clustering Calculator "fast clustering algorithms, including UPGMA and Saitou-Nei neighbor joining" Department of Biological Sciences, University of Alberta;Canada. http://www.biology.ualberta.ca/jbrzusto/cluster.php.

Carvalho, M. Matos, M. & Carnide, V. (2018). Identification of cultivated and wild *Vaccinium* species grown in Portugal. Spanish Journal of Agricultural Research, 16 (3): e07SC01.

Celikel, G. Demirsoy, L. & Demirsoy, H. (2008). The strawberry tree (*Arbutus unedo* L.) selection in Turkey. Sci. Hort, 118: 115–119.

Chesnokov, Y.V. & Artemyeva, A.M. (2015). Evaluation of the measure of polymorphism information of genetic diversity. Agricultural Biology, 50: 571-578.

De Riek, J. Calsyn, E. Everaert, I. Van Bockstaele, E. & De Loose, M. (2001). AFLP based alternatives for the assessment of Distinctness, Uniformity and Stability of sugar beet varieties. Theoretical Applied Genetics, 103:1254-1265.

Debnath, S. C. (2009). Development of ISSR markers for genetic diversity studies in *Vaccinium angustifolium*. Nordic Journal of Botany, 27: 141-148.

Debnath, S.C. (2007). Inter simple sequence repeats (ISSR) to assess genetic diversity within a collection of wild lignonberry (*Vaccinium vitis-idaea* L.) clones. Canadian Journal of Plant Science, 87: 337- 344.

Debussche, M. & Isenmann, P. (1989). Fleshy fruit characters and the choices of bird and mammal seed dispersers in a Mediterranean region. Oikos, 56: 327-338.

Debussche, M. & Isenmann, P. (1989). Fleshy fruit characters and the choices of bird and mammal seed dispersers in a Mediterranean region. Oikos,56: 327-338.

Ercisli, S. Tosun, M. Karlidag, H. Dzubur, A. Hadziabulic, S. & Aliman, Y. (2012). Colorand antioxidant characteristics of some fresh fig (*Ficus carica* L.) genotypes from Northeastern Turkey. Plant Foods for Human Nutrition, 67: 271−276.

Fazenda, P. & Miguel, J. (2013). Identificação de marcadores SSR e de SNPs em medronheiro (*Arbutus unedo* L,) por sequenciação massiva paralela, Instituto superior de Agronomia, Universidade de Lisboa, Spain.

Fazenda, P. Pereira, R. Fonseca, M. Carlier, J. & Leitão, J. (2019). Identification and validation of microsatellite markers in Arbutus tree (*Arbutus unedo* L.). Turk J Agric For, 43: 430-436.

Godinho-Ferreira, P.G. Azevedo, A.M. & Rego, F. (2005). Carta da tipologia florestal de Portugal Continental. Silva Lusit, 13: 1–34.

Godt, M.J.W. Hamrick, J.L. Edwards-Burke, M.A. & Williams, J.H. (2001). Comparisons of genetic diversity in white spruce (*Picea glauca*) and jack pine (*Pinus banksiana*) seed orchards with natural populations. Canadian Journal of Forest Reseaech, 31: 943–949.

Gomes, F. & Canhoto, J. (2009). Micropropagation of strawberry tree (*Arbutus unedo* L.) from adult plants. In Vitro Cell. Dev. Biol. Plant, 45: 72–82.

Gomes, F. Costa, R. Ribeiro, M.M. Figueiredo, E. & Canhoto, J.M. (2012). Analysis of genetic relationship among *Arbutus unedo* L. genotypes using RAPD and SSR markers. Journal of Forestry Research, 23: 4.

Gomes, M.F.F.N. (2011). Strategies for the Improvement of *Arbutus unedo* L. (Strawberry Tree): In vitro Propagation, Mycorrhization and Diversity Analysis. Ph.D. Thesis, Universidade de Coimbra, Coimbra, Portugal.

Govindaraju, D.R. (1988). Relationship between dispersal ability and levels of gene flow in plants. Oikos. 52(1): 31–5.

Gupta, R. Verma, K. Joshi, D.C. Yadav, D. & Singh, M. (2010). Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. Genetic Engineering and Biotechnology Journal, GEBJ-2.

Hagerup, O. (1957). Wind autogamy in Arbutus. Bull Jard Bot. État Bruxelles. 27: 41-47.

Hamdani, A. Bouda, S. Houmanat, K. Outghouliast, H. Razouk, R. Adiba, A. & Charafi, J. (2022): Genetic diversity revealed via molecular analysis of moroccan and foreign plum (*Prunus domestica*; *Prunus salicina*) genotypes from an ex-situ collection. Vegetos, https://doi.org/10.1007/s42535-022-00463-7.

Hamrick, J.L, & Godt, M.J.W. (1990). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahle AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, MA, pp. 43-63.

Hamrick, J.L. & Godt, M.J.W. (1996). Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society: Biological Sciences, 351:1291–1298.

Hamrick, J.L. Godt, M.J.W. & Sherman-Broyles, S.L. (1992). Factors influencing levels of genetic diversity in plant species. New Forest, 6: 95–124.

Kabiri, G. Bouda, S. & Haddioui, A. (2022). Evaluation of genetic diversity and structuration across altitude of walnut (Juglans regia L.) accessions from Morocco using SSR markers. Bulg J Agric Sci, 28(3): 451–458.

Kercher, S.M. & Sytsma, K.J. (2000). Genetic and morphological variation in populations of the rare prairie annual *Agalinis skinneuiana* (Wood) Britton (*Scrophulariaceae*). Natural Areas Journal, 20: 166-175.

Levin, D.A. (1984). Immigration in plants: an exercise in the subjunctive. In: Dirzo R and Sarukhan J (eds) Perspectives on plant population biology. Sinauer, Sunderland, Massachusetts, USA, pp. 242-260.

Lopes, L. Sáa, O. Pereiraa, J.A. & Baptista, P. (2012). Genetic diversity of Portuguese *Arbutus unedo* L. populations using leaf traits and molecular markers: An approach for conservation purposes. Scientia Horticulturae, 142: 57-67.

Mekhfi, H. El Haouari, M. Bnouham, M. Aziz, M. Ziyyat, A. & Legssyer, A. (2006). Effects of extracts and tannins from *Arbutus unedo* L. leaves on rat platelet aggregation. Phytotherapy Research, 20:135–139.

Mereti, M. Grigoriadou, K. Leventakis, N. & Nano, G.D. (2003). In vitro rooting of strawberry tree (*Arbutus unedo* L.) in medium solidified by peat–perlite mixture in combination with agar. Acta Horticulturae, 616: 207–210.

Mesléard, F. & Lepart, J. (1991). Germination and seedling dynamics of *Arbutus unedo* and Erica arborea on Corsica. J Veg Sci, 2:155-164.

Métais, I. Aubry, A. Hamon, B. & Jaluozot, R. (2000). Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). Theor Appl Genet, 101: 1207-1214.

Ouinsavi, C. & Sokpon, N. (2010). Morphological Variation and Ecological Structure of Iroko (*Milicia excelsa* Welw. C.C. Berg) Populations across Different Biogeographical Zones in Benin. International Journal of Forestry Research, 6: 583-596.

Pallauf, K. Rivas-Gonzalo, J.C. Castillo, M.D, Cano, M.P. & Pascual-Teresa, S. (2008). Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. Journal of Food Composition and Analysis, 21(4): 273–281.

Powell, W. Morgante, M. Andre, C. Hanafey, M. Vogel, J. Tingey, S. & Rafalski, A. (1996). The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Pradeep, M. Sarla, N. & Siddiq, E.A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

Prevost, A. & Wilkinson, M.J. (1999). A new system of comparing PCR primers applied to ISSR fngerprinting of potato cultivars. Theoretical Applied Genetics, 98(1): 107–112.

Reddy, M.P. Sarla, N. & Siddiq, E.A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128 (1): 9–17.

Reddy, M.P. Sarla, N. Neeraja, C.N. & Siddiq, E.A. (2000). Fourth International Rice Genetics Symposium, IRRI, Philippines. Abstracts, pp. 212.

Reis, M.S. (1996). Dinâmica da movimentação dos alelos: subsídios para conservarção e manejo de populações naturais de plantas. Brazil J Genet, 19: 37-47.

Ribeiro, M.M. Piotti, A. Ricardo, A. Gaspar, D. Costa, R. Parducci, L. & Vendramin, G.G. (2017). Genetic diversity and divergence at the *Arbutus unedo* L. (Ericaceae) westernmost distribution limit. PLoS ONE, 12(4): e0175239.

Rodriga de Sà, O. (2010). Caracterizaçäo Morfologica, Molecular e Quimica de *Arbutus unedo* L. com vista à selecçäo de genotipos de superior qualidade. Instituto Plitécnico, Escola Superior Agräria de Bragança, Espagne.

Rodriga de Sà, O. Pereira, J.A. & Baptista, P. (2011). Optimization of DNA extraction for RAPD and ISSR analysis of *Arbutus unedo* L. leaves. Int J Mol Sci, 12: 4156-4164.

Roldan-Ruiz, I. Dendauw, J. Van-Bockstaele, E. Depicker, A. & De Loose, M. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding, 6:125–134.

Rossetto, M. Weaver, P.K. & Dixon, K.W. (1995). Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (*Proteaceae*). Molecular Ecology, 4: 321–329.

Ruiz-Rodriquez, B.M. Morales, P. Fernandz-Ruiz, V. Sánchez Mata, M.C. Cámara, M. Díez Marqués, C. Pardo De Santayana, M. Molina, M. & Tardío J. (2011). Valorization of wild strawberry tree fruits (*Arbutus unedo* L) through nutritional assessment and natural production data. Food Research International, 44: 1244–1253.

Saghai-Maroof, M.A. Soliman, K.M. Jorgensen, R.A. & Allard, R.W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, 81: 8014–8018.

Serçe, S. Ozgen, M., Torun, A.A. & Ercisli, S. (2010). Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne* L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey. Journal of Food Composition and Analysis, 23: 619–623.

Shimada, T. Hayama, H. Haji, T. Yamaguchi, M. & Yoshida, M. (1999). Genetic diversity of plums characterized by random amplified polymorphic DNA (RAPD) analysis. Euphytica, 109:143–147.

Slatin, M. (1987). Gene flow and geographic structure of natural populations. Science, 236: 787–792.

Sorkheh, K. Shiran, B. Gradziel, T.M. Epperson, B.K. Martinez-Gomez, P. & Asadi, E. (2007). Amplified fragment length polymorphism as a tool for molecular characterization of almond germplasm: genetic diversity among cultivated genotypes and related wild species of almond, and its relationships with agronomic traits. Euphytica, 156: 327-344.

Sorkheh, K. Shiran, B. Kiani, S. Amirbakhtiar, N. Mousavi, S. Rouhi, V. Mohammady, D.S. Gradziel, T.M. Malysheva-Otto, L.V. & Martínez-Gómez, P. (2009). Discriminating ability of molecular markers and morphological characterization in the establishment of genetic relationships in cultivated genotypes of almond and related wild species. Journal of Forestry Research, 20: 183-194.

Takrouni, M.M. & Boussaid, M. (2010). Genetic diversity and population's structure in Tunisian strawberry tree (*Arbutus unedo* L.). Scientia Horticulturae, 126: 330–337.

Takrouni, M.M. Ali, I.B.H. Messaoued, C. & Boussaid, M. (2012). Genetic variability of Tunisian wild strawberry tree (*Arbutus unedo* L.) populations interfered from isozyme markers. Sci. Hort., 146: 92–98.

Torres, J.A. Valle, F. Pinto, C. Garcia-Fuentes, A. Salazar, C. & Cano, E. (2002). *Arbutus unedo* L. communities in southern Iberian *Peninsula mountains*. Plant Ecol, 160: 207–223.

Vidal, J.R. Coarer, M. & Defontain. (1999). A Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers. Euphytica, 109:161–172.

Vidrih, R. Hribar, J. Prgomet, Z. & Poklar-Ulrih, N. (2013). The physicochemical properties of strawberry tree (*Arbutus unedo* L.) fruits. Croatian Journal of Food Science and Technology, 5: 29–33.

Wright, S. (1978). Evolution and the genetic of population, variability within and among natural populations, vol. 4. Chicago: University of Chicago Press. p. 213–20.

Zaefizadeh, M. & Goliev, R. (2009). Diversity and relationships among durum wheat landraces (Subconvars) by SRAP and phenotypic marker polymorphism. Research of Biology & Scientia, 4: 960-966.

Zhang, X. Zhang, Y. Yan, R. Han, G. Hong, F. Wang, J. & Cao, K. (2010). Genetic variation of white clover (*Trifolium repens* L.) collections from China detected by morphological traits, RAPD and SSR. Afr J Biotechnol, 9(21): 3032-3041.

Zietkiewicz, E. Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genetics, 20: 176–183.

Zitouni, H. Fauconnier, M.L. Hssaini, L. Ouaabou, R. Viuda-Martos, M. Hernández, F. Ercisli, S. Ait Haddou, L. Zerhoune, M. & Hanine, H. (2022). Phenols, Volatile Compounds, Organic Acids and Antioxidant Activity of Strawberry Tree (*Arbutus Unedo* L.) Fruits Belonging to Five Genotypes Growing in Morocco. International Journal of Fruit Science, 22(1): 414-437.

Zitouni, H. Fauconnier, M.L. Hssaini, L. Viuda-Martos, M. Hernández, F. Ercisli, S. Zerhoune, M. Elothmani, D. Ennahli, S. & Hanine, H. (2021). Strawberry Trees (*Arbutus unedo* L.) Naturally Grown in Morocco: A Combined Study Using Headspace Solid Phase Microextraction Coupled with GC-MS and Physico-Morphological Screening. ACS Food Science & Technology, 1(5): 943-959.

Zitouni, H. Hssaini, L. Zerhoune, M. Ourradi, H. Viuda-Martos, M. Hernández, F. Ercisli, S. & Hanine, H. (2020). Phytochemical components and bioactivity assessment among twelve strawberry (*Arbutus unedo* L.) genotypes growing in Moroccan using chemometrics. Foods, 9: 1345.

Ziyyat, A. Mekhfi, H. Bnouham, M. Tahri, A. Legssyer, A. Hoerter, J. & Fischmeister, R. (2002). *Arbutus unedo* induces endothelium-dependent relaxation of the isolated rat aorta. Phytoterapy Research, 16 : 572–575.

Marić, D. (2023): Changes in the habitat and nest site distribution for two Lanius species in karst poljes (Bosnia and Herzegovina). Agriculture and Forestry, 69 (4): 157-172. doi:10.17707/AgricultForest.69.4.10

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CHANGES IN THE HABITAT AND NEST SITE DISTRIBUTION FOR TWO *LANIUS* SPECIES IN KARST POLJES (BOSNIA AND HERZEGOVINA)

SUMMARY

This study presents the first comparative analysis for the selection of nesting sites of two shrike species in habitats that were changed after total emigration of the human population. In this area, two shrike species are present (Red-backed Shrike - *Lanius collurio* and Lesser Grey Shrike - *Lanius minor*). The selection of nesting sites of two shrike species (breeding population) between two periods was compared: before and after the Balkan civil conflict in three karst poljes (karst fields), municipality of Bosansko Grahovo. Data was collected in 1974–1991 and in 2001–2022 in three karst poljes at Bosansko Grahovo. In total 998 nests of Red-backed Shrikes and Lesser Grey Shrikes (822 and 176 nests respectively) were analysed. The vast majority (67.0% in first and 40.2% in second period) of nests of Red-backed Shrikes were located on the *Crataegus monogyna* (total 17 species of plants). The Lesser Grey Shrike had the maximum number of nests (over 40%) placed on the two trees/shrubs (*C. monogyna* and *Prunus domesticus*) in the first period, and additionally in *Sambucus nigra* (17.9% of nests) in the second period. Significant differences were found in the selection of nesting sites between these two species and between two periods. Only five trees of nest locations for both species were registered. The Red-backed Shrike prefers lower shrubs (0.8–2.8 m) and Lesser Grey Shrike 3.5–10 m high shrubs/trees. It was confirmed that the presence of suitable nest sites is one of the most important limiting factors determining the distribution of two species in karst poljes.

Keywords: nest site distribution, Red-backed Shrike, Lesser Grey Shrike, karst poljes, Bosnia and Herzegovina

INTRODUCTION

Abandonment of the village and thus arable land leads to changes in the natural environment resulting in varying habitats, many of which are extreme for birds, for example *Emberiza citrinella* or *Passer domesticus*, however, for some

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species this had a favourable effect (Marić 2022b). Some of the species whose abundance increased after the mass emigration of the human population (during the war in Bosnia and Herzegovina - Nikolić 2021) are *Lanius* spp. In addition to villages, the number of these species increased in other habitats where they had suitable places for nesting (Marić 2022b, pers. obs.) However, shrike species have been showing a marked decline in their breeding population for the last third of the last century in almost all of Europe (Yosef 1994, Krištín *et al*. 2000, Brambilla *et al.* 2009). According to the European regulations, they are protected due to their declining across the breeding range (Tucker *et al*. 1994, Brambilla *et al.* 2009, Sfougaris *et al*. 2014, etc.). Generally, farmland bird species are in decline across the whole of Europe (Tucker and Evans 1997, Gregory *et al.* 2008, Sutcliffe *et al.* 2015). Among the bird species which depend on agriculture management are Shrikes and it represents an important group of farmland bird species which inhabit open habitats.

According to Marić (2022a) *L. collurio* inhabits six different habitat types, and *L. minor* three in the Grahovo's poljes. According to Cramp (1994) and Yosef (2008) *L. minor* inhabits open habitat with plenty of scattered or grouped trees, and fewer bushes, requires presence of features offering perches, shade and accessible food. Breeding habitats in Europe included extensively managed orchards, as well as vineyards and meadows, tall trees necessary for nesting. Prefers open or disturbed lowland and hilly areas to 700 m, rarely to 900 m in C. Europe. Need for drier and sunnier conditions than those tolerated by other European shrikes possibly connected with more specialized diet or large insects. Red-backed Shrikes (*L. collurio*) have favoured shrubby cattle grazed pastures or other habitats with sustained low vegetation allowing good visibility of ground living beetles (Brandl *et al.* 1986, Olsson 1995a). The majority of the population breeds in semi-open habitats created or maintained by human activities (Lefranc and Worfolk 1997). The Red-backed Shrike is breeding in most parts of Europe. According to Block and Brennan (1993) the breeding habitat is selected based on nesting requirements, whereas the non-breeding habitat use is strongly associated with food abundance. Red-backed Shrikes are particularly sensitive to both agricultural intensification and land abandonment (Brambilla *et al*. 2007). Both shrike species occur in the karst poljes (karst fields) of Bosnia and Herzegovina (Obratil 1984, 1987, 2006, Kotrošan *et al*. 2013, Puzović *et al.* 2019). Scarce data on biology of shrike species can be found scattered in the literature. Studying the reproductive performance of bird species in Bosnian karst poljes with different management regimes is urgently needed to understand their impact on avian populations.

In the present study, analysed nest site distribution in the two *Lanius* spp. in three karst poljes in the Western part of Bosnia and Herzegovina. In the present study, analysed nest site distribution in the two *Lanius* spp. in three karst poljes in the Western part of Bosnia and Herzegovina. In this paper, research was done on which changes in the vegetation influenced the choice of nesting sites of these species. The comparative analysis is between the period 1974–1991 (there were no significant population movements) and the period 2001–2022, five years after the total emigration of the human population. To the best of our knowledge, no study has considered shrike nesting in in the area of karst poljes. Nest site selection is generally considered an important component of habitat selection by birds (e.g. Hildén 1965). Some of the most commonly used parameters are tree species, the height of the tree, the nest's height above the ground (Marić 2023).

MATERIAL AND METHODS

Study area

The study area, three karst poljes are situated in the central part of the municipality of Bosansko Grahovo (=B. Grahovo). B. Grahovo is situated in the west part of Bosnia and Herzegovina (B&H) (Figure 1) and it covers 780 km^2. This area is characterized by high diversity of habitats (for detailed information and habitat description see Marić 2022a). There are 4 karst poljes: Livanjsko polje and three Grahovo poljes (790–860 m a.s.l., 29 km length and width 2–4 km=80 km^2). They are situated between 16°18'00" and 16°27'00" of eastern geographical longitude and 44°08'00" and 44°18'00" of northern geographical latitude. The area holds one of the most representative traditional and low-intensity agricultural systems in the karst poljes.

The main habitats of this area are agricultural land, meadows and pastures. Arable fields cover less than 20% of total land cover, and are situated only in central part of poljes. The slopes of the hills, around poljes, are covered with pastures and various succession stages of forestation. The afforestation of the rocky hills and the pasture around karst poljes was done with pine (*Pinus* sp.) and covers an area of about 10 km^2 (pers. obs.). Also, the marginal parts of karst poljes are naturally overgrown with pines and several deciduous species. Arable lands are small sized arable fields (mainly fodder and cereals, including wheat and oat).

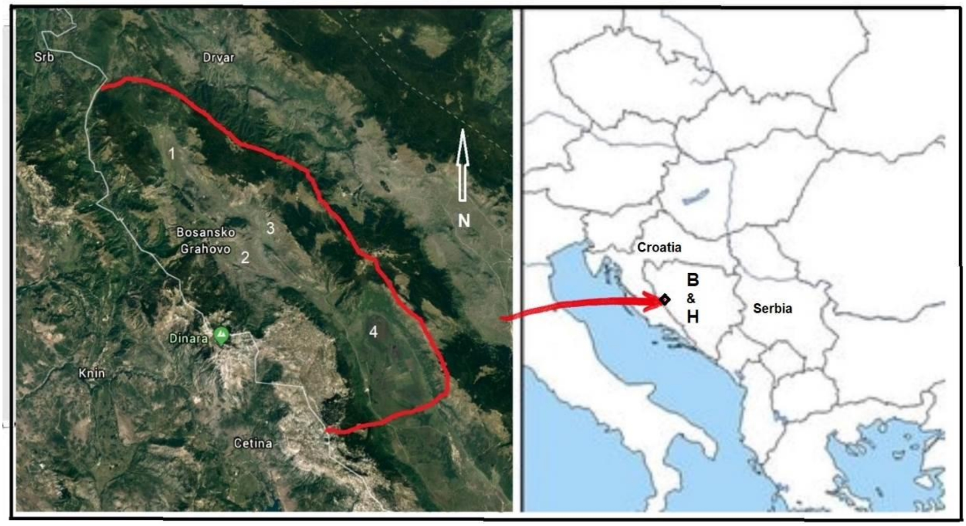

Figure 1. The area of the municipality of Bosansko Grahovo, red line border between the municipalities and white line borders between the states. B&H=Bosnia and Herzegovina, 1) Resenovačko polje, 2) Pašića polje, 3) Marinkovci polje, 4) Livanjsko polje

Arable fields recently abandoned cultivations and pastures progressively covered by shrubs and small trees. There are broadleaved forests (secondary woods derived from tree recovery over abandoned land), then forests along the banks of creeks and rivers, habitat type is similar to 91E0, according to Natura 2000 code. These forests (small groves of alder, willows and poplar) are along the banks of sinking rivers (the Struga and the Korana). Around poljes there are rocky habitats, orchards and small villages. Fruit species are dominated by plums, apples, pears and cherries (deciduous fruit trees), and in the village grow trees, such as *Tilia* spp., and shrubs such as *Cornus mas*, *Rosa* spp., *Crataegus monogyna*, *Sambucus nigra*, *Prunus spinosa*, etc. (see Marić 2022a, 2022b).

Nesting of *L. collurio* was found in 6 types of habitats: 1) forests along the banks of creeks and rivers, 2) high shrub deciduous and evergreen bushes (up to 7-10m) or forests with large clearings, degraded forests in different degradation stages, 3) deciduous bushes: bushes, shrubs of low trees and shrubs (mostly up to 3m high) with large clearings (20-70%), 4) dry calcareous grasslands, from karst poljes to the montane zone, (in habitats 3 and 4, afforestation has been carried out in the last 20 years), 5) meadow and agricultural land (arable fields, grasslands and wet meadows of karst poljes), 6) settlements (villages and town) with orchards.

Nesting of *L. minor* was found in three types of habitats: 1) forests along the banks of creeks and rivers, 2) meadow and agricultural land (arable fields, grasslands and wet meadows of karst poljes), 3) settlements (villages and town) with orchards (details see in Marić, 2022 a).

Field methods, sampling strategy

Our study was carried out at three karst poljes, at B. Grahovo, in the Pašića, Marinkovci and Resanovačko polje, during the two periods: first period before the Balkans civil conflict (1974–1991), and second period, 5 years after the Balkans civil conflict (2001–2022). Studies of the shrikes in this area of the period 1992–2000, was not possible due to Civil War and post-war instability. The size of the study area was approximately 80 square kilometers. *Lanius* spp. were censused at six habitats characterized by different land-use (see Marić 2022a) and representing the main landscape types found in the area. All these habitats were included in our study plots, in proportion to their relative extension in the study area.

Two methods were used in bird surveying: first method - line transect or point counts (see Marić 2022b), second method - systematically searched for nests across each habitat (localities), using visual and auditory cues (Martin and Geupel 1993). Both methods are well suited to monitoring shrikes, as they are sedentary, conspicuous and mono-territorial during the breeding season. Transects were carried out in the mornings and the targeted search for nests was carried out throughout the day. For each habitat, the nests were counted along transects (see Marić 2022b) or systematic search of nests. After the identification of *Lanius* species, their nests were detected by observing the birds heading towards their nests i.e. adults carrying food and nest material or exhibiting behaviours typically elicited when predators are near nests. Nests were discovered at various developmental stages, including nest building, incubation and brood rearing. Most nests were found during nest building or egg laying periods. A 15 cm diameter mirror mounted on a telescopic pole was used to estimate eggs or chickens, but also to measure the height of nests, only in the second period (measurement error=circa 10cm).

Nests were discovered during the morning hours (between 6 and 12 o'clock) and in the afternoon (between 4 and 7 o'clock). Fieldwork took place between 20th May and the the end of July, i.e. bird surveys were conducted during the breeding season. The localities were surveyed under favourable weather conditions (without heavy rain, mist and strong wind). Censuses were not carried out in wet or strong windy (> Force 5 on the Beaufort scale) weather.

Statistical analysis

Descriptive statistics were carried out and number of nests were tested (number or %). In order to test differences between periods the z-test was conducted for all tree species separately, i.e. share of the total number of nests %. The differences between periods for all tree species (in total) was calculated from the hypothetical ratio (50:50) and were tested by means of test - χ^2 (Sokal and Rohlf 1981). All statistical tests were independent and two-tailed. The statistical analyses were performed using STATISTICA software package. Results are considered significant if $P \leq 0.05$.

RESULTS

During the research period, 15 to 20 pairs of *L. minor* and over 150 pairs of *L. collurio* were registered as nesting in the research area (three karst fields) during one year. A total of 998 nests were located for these two species during 40 breeding seasons, 822 nests of *L. collurio* and 176 of *L. minor* were observed during the present work. The analysis of nest site distribution of two shrike species' observations in period 1974–1991 and 2001–2022 from three karst poljes from the municipality of B. Grahovo are shown in Table 1. More than 20 tree species are commonly selected by *Lanius* spp. for nesting (a total of 25 species). Red-backed Shrike used 17, and Lesser Grey Shrike 14 tree species (Table 1) as nest sites in three Grahovo karst poljes. No significant differences (χ^2) were found in the total number of nests and selected trees between the studied periods ($P>0.05$).

Table 1. Tree and bush species used as *Lanius* nest sites in the six habitats in Grahovska polja. Given are number of nests N, share of the total number of nests-percentage (%), z-test and significance differences (ns – no significant, P<0.05 #, 0.01 ##, 0.001 ###)

Species	*Lanius collurio*			*Lanius minor*		
	Periods			Periods		
	1974–1991 N (%)	2001–2022 N (%)	*z*	1974–1991 N (%)	2001–2022 N (%)	*z*
Acer sp.	-	-	-	2	1	ns
Alnus glutinosa	-	-	-	1	3	ns
Amelanchier ovalis	-	3	-	-	-	-
Carpinus betulus	1	3	ns	1	3	ns
Corilus avelana	24	21	ns	-	-	-
Cornus mas	12	27	5.8#	-	-	-
Cornus sanguinea	5	24	6.2	-	-	-
Crataegus monogyna	246 (67.0)	188 (41.3)	7.8###	26 (35.1)	21 (19.8)	ns ns
Fraxinus sp.	1	2	ns	1	2	-
Malus sylvestris	2	18	12.8###	-	-	-
Malus pumila	-	-	-	3	5	ns
Ostria carpinifolia	7	14	ns	-	-	-
Pinus niger	-	3	-	-	-	-
Pirus communis	-	-	-	1	7	ns
Populus sp.	-	-	-	2	2	ns
Prunus cerasus	-	-	-	3	1	ns
Prunus domesticus	4	23	13.4###	16 (21.6)	21 (19.8)	ns
Prunus mahaleb	3	4	ns	-	-	-
Prunus spinosa	33 (9.0)	47 (10.3)	ns	3	8	-
Rhamus frangila	2	2	ns	-	-	ns
Robinia pseudacacia	-	-	-	6	9	ns
Rosa spp	21	24	ns	-	-	-
Salix alba	-	-	-	4	3	ns
Salix purpurea	2	15	9.9##	-	-	-
Sambucus nigra	4	36 (7,9)	4.4###	2	19 (17.9)	3.04##
Total	**367**	**455**		**71**	**105**	

DISCUSSION

In recent decades, changes in the structure of the landscape at the local and regional scale in many areas of Bosnia and Herzegovina have been observed. In general, such changes have influenced the composition of biodiversity in those/such habitats then changes in ecology, phenology and diversity of species assemblages (e.g. Chamberlain *et al.* 2000, Marić 2022b, etc.). According to Baur *et al*. (2006) and Queiroz *et al*. (2014) the effects of land abandonment on biodiversity are complex and can vary regionally and between taxa. Also, results show contrasted responses for different bird groups, with “loser” and “winner” species (Phalan *et al*. 2011, Teillard *et al.* 2015). Abandonment of the villages in some karst poljes in Bosnia and Herzegovina led to changes in bird populations, some have left (disappeared) the area, some have reduced abundance, for example *Emberiza citrinella* or *Passer domesticus*, and some had a favourable effect (Marić 2022b). Some of the species whose abundance increased after the mass emigration of the human population are *Lanius* spp. In addition to villages, the number of these species increased in other habitats where they had suitable places for nesting (pers. obs.).

In this paper, the focus is on two species of birds typical of agricultural landscapes, *L. collurio* and *L. minor*. Habitat and nest site preferences of shrikes have not yet been studied in karst poljes in Bosnia and Herzegovina. This study presents the first comparative analysis of the selection of nesting sites of both shrikes in habitats that were changed after the total emigration of the human population. It therefore provides basic indications for the conservation of this species in this important part of its distribution range, i.e. in karst poljes. Results showed that both species of shrikes react positively to changes in the habitat.

The biggest changes are evident in the settlements and orchards habitats, this is because this type of habitat in many villages is now without human population, and thus without domestic animals. The lack of people affected the orchards because they are not cultivated, and the disappearance of the sheep resulted in intensive growth of bushes and an increase in the presence of some plants as black elder. Black elders also appear inside demolished buildings. The lack of domestic livestock, primarily sheep, enabled the intensive growth of various types of shrubs in all habitats, especially in the deciduous bushes habitat. If we add to this that intensive afforestation was carried out, it is clear that the changes in the habitats of these two species are evident and permanent.

The Red-backed Shrike inhabits the most habitat types of all species registered in the research area and are significantly more numerous in sympatry than *L. minor* (Marić 2022a). Also, according to Marić (2022b), both species of shrikes significantly increased in abundance in the three studied habitats in the Pašića poljes after the exodus of people. This paper shows how these changes influenced the selection of nesting sites for these species in three karst fields in the municipality of B. Grahovo. The nests of Red Backed Shrikes were recorded in six observation habitats while the Lesser Grey Shrike in only three observation habitats. Nest-site selection is a key component of habitat selection by birds (Hildén 1965), with important consequences for survival and reproduction of individuals (Cody 1985).

Lesser Grey Shrike - *Lanius minor*

An analysis of 176 records of nest sites used by Lesser Grey Shrikes from 1974 to 2022 showed that *Crataegus* and tree fruits were the most preferred species in both periods, followed by *S. nigra*. The analysis also showed that this species did not nest in underbushes and forest clearings and pastures. Contrary to this, according to Ajder and Baltag (2017) the Lesser Grey Shrikes select also pastures for breeding. The same preference of trees was recorded also in Greece (Sfougaris *et al*. 2014), Hungary (Lovászi *et al*. 2000), Romania (Moga *et al.* 2010) and Slovakia (Krištín 1995).

(A) Natural succession of pastures after the exodus of people

(B) Pastures after forestation

(C) A village with many black elders

(D) Black elders sprouted from the collapsed buildings

(E) A nest in a black elder bush at a 0.8 m height

(F) *L. collurio*, nest in *Salix purpurea*

(G) Settlements with electric wires are a habitat for *L. minor*

(H) *L. minor*, nest in a solitary tree in the karst field

Figure 2. Panoramic view on the studied area of karst poljes and selected habitats

In this area, the deciduous trees on the pastures are still of small height, so this is probably the main reason for the absence of this species on the pastures. Tall pines appeared in part of the pasture, either naturally or afforestation with those species was carried out. Only *L. collurio* nests in them, but not *L. minor*, although these trees have grown to the optimal heights preferred by this species (4–10 m in this study). The increase in the occupancy of fruit species is the result of these fruit species not being pruned and cultivated (absence of people), and the increase in *S. nigra* due to the increase in its abundance also due to the lack of people and domestic animals. From each collapsed object (house or building) black elders emerged, which grew up to 7 m at the end of the studied period (Figure 2 C, D pers. obs.). The increase in numbers and growth of black elder is significant in the second period. This provided many potential nesting places preferred by the Lesser Grey Shrike. There are many orchards which are the main breeding place of Lesser Grey Shrikes and *Prunus domesticus* mostly preferred (ca. 20.0%). This is explained by the fact that branches of *P. domesticus* are most favourable for nest location. In addition, this tree species is the most common in the villages in this area (more than 70% of all tree species). The structure of these trees offers better conditions for nesting than others. Other species also nest on the most abundant trees (Sakhvon and Kövér 2020, Marić 2023).

More than 10 tree species are commonly selected by *L. minor* for nesting, *C. monogyna* (26.6%), *P. domesticus* (22.5%) are mostly preferred in the first period, and *C. monogyna* (19,8%), *P. domesticus* (19.8%), *S. nigra* (17.9%) in the second period. *S. nigra* rapidly increased in number after the village was abandoned and this provided many potential nesting places preferred by the *Lanius* spp.

Finally, the electric poles and wires, which serve as hunting perches in the breeding area, might be another factor effecting their habitat preferences and tree selection near electric poles. The pattern found in this study is not confirmed by other studies from Europe.

The predominant use of poplars for nest building was noted also by Horváth (1959) and Lovászi *et al*. (2000). In the study conducted by Lovászi *et al*. (2000), beside poplars, four other species were also used in nest building, but to a lesser degree. Krištín (1995) noted that 97% of the observed nests were built in fruit trees and similarly noted Wirtitsch *et al.* (2001), in a study from central Slovakia. The authors did not record a clear preference for one kind of fruit tree, some of the species (i.e. apple) were used according to their availability while others not. Our results and the above-mentioned studies suggest that *L. minor* may show a wide preference for microhabitats. These differences are probably due to the specificity of the vegetation of karst poljes.

L. minor nests on tall trees up to 20 m (Peterson *et al*. 1968), and according to Krištín (1995) at heights of 5 to 24 m – average 8.53 m. According to the data of this paper, this species nests at much lower heights (4–10 m) because there are no tall trees in the habitats it inhabits, except for Linden. Also, according to the data of the aforementioned authors, this species was not selected for nesting, which indicates that it is not suitable for building nests, and Magpies in the Zeta river valley did not build nests on Linden trees (Marić 2023).

Red Backed Shrike - *Lanius collurio*

The Red-backed Shrike inhabits the most habitat types of all species registered in the study area and are significantly more numerous in sympatry than *L. minor*. The Red-backed Shrike preferred shrubs and open areas e.g. pasture or agricultural land i.e. habitats with low vegetation and a low concentration of shrubs in (Marić 2022a). This species used 17 types of trees for nesting. According to Kosiński (2001) 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. This plasticity of nest placement was previously observed in *L. collurio* (Lislevand 2012) but in other passerines (Wilson and Cooper 1998, Marques *et al*. 2002, Lomáscolo *et al*. 2010) and in Columbiformes (Hanane 2012, 2014a).

Bushes presenting good perches for insect hunting nearly always also provide good nesting sites. In the study area, mostly nests this species were found in thorny shrubs and clearly preferred hawthorns. Over 2/3 of all nests in the first period and over 40 in the second were found in these shrubs. Some other authors also pointed to the Red-backed Shrike's preference for thorny or prickly species, such as hawthorn, plum tree (*Prunus* sp.) or blackberry (*Rubus* sp.) (Kuźniak 1991, Nikolov 2000, Baláž 2007). In these studies, nests were not found in blackberry (*Rubus* sp.), which some authors state as a suitable place for nesting (e.g. Svendsen *et al*. 2015). In the researched area, this species is rare and grows low, usually up to 50 cm, which is a possible reason that *L. collurio* does not nest in it. In addition to hawthorn, a considerable number of nests were found in Rosa spp. and *Prunus spinosa* in both periods. Hawthorn and these two species make up over 80% of nesting hosts in the first period, and over 56% in the second. According to Olsson (1995b) about half of all nests (50.2%) were built in dog rose *Rosa canina*, blackthorn *Prunus spinosus* and blackberry *Rubus fruticosus*. Also and other authors (Olsson 1995b, Farkas *et al.* 1997, Campos and Lizarraga 2000, Söderström 2001) found that Red-backed Shrike uses spiny scrub as nest sites. Large, dense, thorny, species with dense leaf cover often make nests invisible to, and unapproachable by, humans and possibly other visually oriented nest predators. Choice of such bush type can be an adaptation, since it reduces the possibility of penetrating the inner bush by potential predators destroying broods (Polak 2012).

This is contrary to other studies which found that Red-backed Shrikes does not selectively choose specific plant species, but simply use the dominant plant species in the breeding area (Holan 1995, Söderström 2001). Goławski (2007) found the preferences of this species for nesting in pear trees (*Pyrus communis*) and black elder, which were relatively rare in his research area. Nesting in black elder is reported by Kuźniak (1991) and Horváth *et al.* (2000). In this study the Red-backed Shrike in three karst poljes frequenlently used black elder only in second period. The more significant use of this species in the second period than the first (z=4.4) is due to the significantly higher abundance of this species in abandoned villages. The black elder is present in all demolished houses, barns, etc. (Figure 2C, D), and it is much more numerous around gardens and orchards in the second period. This provided many potential nesting places preferred by the *Lanius* spp. A high plasticity of shrikes in nest site selection was noted by Jakober and Stauber (1981), who emphasized that nest site selection is influenced by local habitat conditions. The specific selection of trees in this area is due to the specificity of the vegetation of karst fields.

The Red-backed Shrike also used fruit species (plum and wild apple) in considerable numbers, significantly more in the second period (z≈13). Selection of fruit species for nesting is reported by Žolner (1983) and Goławski (2007). The preponderance of nests in this type of trees (plum tree) is partly explained by it being the most common fruit species in the area (more than 80%, pers. obs.). Also, another difference is that the Red-backed Shrike used *Pinus* spp. for nesting in the second period (not in the first). The use of conifers for nesting is mentioned by several authors (Kuźniak 1991, Olsson 1995b, Pedersen *et al*. 2011), but mainly for the area of Northern Europe. *Pinus* spp. was a common tree species planted in the 21th century in many sites, ie. on abandoned pastures. In the next 10 to 20 years, these afforestations will allow an increase in the number of *L. collurio*, but with the increase of *Pinus* spp. a forest will be created, so this species will lose large areas for nesting, and thus for survival. According to Zhang *et al*. (1994) breeding is the most important part of bird life, since a success of breeding can directly affect population dynamics and the continuity of species.

Average height of nests from the ground was higher than that of certain other European populations (Olsson 1995b, Nikolov 2000, Tryjanowski and Sparks 2001, Lislevand 2012). Elsewhere, Red-backed Shrikes placed their nests higher than found in the current study (eg. Denac 2003 or Baláž 2007). The height of nests in the study area mostly did not differ from that reported by other authors in Central Europe (Kuźniak 1991, Goławski and Mitrus 2000, Pedersen *et al*. 2011, Polak 2012, etc). Also, the highest nests in the study area did not differ from that reported by many authors (eg. Olsson 1995b, Baláž 2007, Lislevand 2012, Arslan Şahin *et al*. 2016 etc). Only in a few populations the nest height was 3.5 to 4 m (Pedersen *et al*. 2011, Polak 2012). According to Kuźniak (1991) the highest nests were built in pines at heights of 3.5 to 4 m but the majority of nests (73.2%) were built at a height of 0.7 to 1.8 m. The height of nests variability in different populations may be due to habitat type or tree differences in breeding areas.

CONCLUSIONS

To fill the gap in our knowledge on srikes’ nesting places, in this paper the nests of two species nesting in karst fields were characterized. In addition to fulfilling these descriptive purposes, the species' nesting site preferences were assessed for the first time in six habitats, which differ in vegetation structure and the availability of different nesting niches. Differences in patterns observed between these habitats are discussed and results compared with other studies.

Generally, the results showed that the species-habitat relationships observed in our study were similar to those reported in the literature.

The selection of nest sites of two species of birds between two periods was compared: before and after Balkans civil conflict in thre karst poljes, the municipality of Bosansko Grahovo. In both species, differences were found between the two studied periods. This might relate to the less intensive agriculture and reduced anthropogenic disturbance generally found in second period in our study area.

Further studies on the consequences of choosing different nest sites in the area of karst poljes and characteristics of nest site choice in different habitats and their impact on the daily survival rates and thus the reproductive success are required to enhance our understanding of the processes influencing selection of nest placement by the Shrikes in the agricultural man-made environment when it is completely abandoned.

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REFERENCES

Ajder, V. & Baltag, E. S. (2017). Factors affecting occurrence of Red-backed Shrike (*Lanius collurio*) and Lesser Grey Shrike (*Lanius minor*) in low-intensify agriculture areas from Eastern Europe. – Polish Journal of Ecology 65(2): 285–294. DOI: 10.3161/15052249PJE2017.65.2.010

Arslan, Şahin, N., Turan, S. L. & Ayaş, Z. A. (2016). Breeding biology of the Red-backed Shrike, *Lanius collurio*, in the Kızılırmak Delta in the north of Turkey. – Turkish Journal of Zoology 40(4): 480–488. DOI: 10.3906/zoo-1509-8

Baláž, M. (2007). On the breeding biology of the Red-backed Shrike (*Lanius collurio*) in the newbreaks of SW Slovakia. – Acta Zoologica Universitatis Comenianae 47(1): 35–39.

Baur, B., Cremene, C., Groza, G., Rakosy, L., Schileyko, A. A., Baur, A., Stoll, P. & Erhardt, A. (2006). Effects of abandonment of subalpine hay meadows on plant and invertebrate diversity in Transylvania, Romania. – Biological Conservation 132(2): 261–273. DOI: 10.1016/j.biocon.2006.04.018

Block, W. M. & Brennan, L. A. (1993). The habitat concept in ornithology. Theory and applications. – In: Power, D. M. (ed.) Current Ornithology 11: 35–91. – Plenum Press, New York, DOI: 10.1007/987-1-4757-9912-5-2

Brambilla, B., Rubolini, D. & Guidali, F. (2007). Between land abandonment and agricultural intensification: habitat preferences of Red-backed Shrikes *Lanius collurio* in low-intensity farming conditions. – Bird Study 54(2): 160–167. DOI: 10.1080/00063650709461471

Brambilla, M., Casale, F., Bergero, V., Crovetto, G. M., Falco, R., Negri, I., Siccardi, P. & Bogliani G. (2009). GIS-models work well, but are not enough: habitat preferences of *Lanius collurio* at multiple levels and conservation implications. – Biological Conservation 142(2): 2033–2042. DOI: 10.1016/j.biocon.2009.03.033

Brandl, R., Lübcke, W. & Mann, W. (1986). Habitatwahl beim Neunt er *Lanius collurio* [Habitat selection in the Red-backed Shrike *Lanius collurio*]. – Journal für Ornithology 127(1): 69–78. DOI: 10.1007/BF01641444 (in German with English Summary)

Campos, F. & Lizarraga, A. (2000). The Red-backed Shrike (*Lanius collurio*) - an indicator of climate and vegetation type? – Ring 22: 59–64.

Chamberlain, D. E., Fuller, R. J., Bunce, R. G. H., Duckworth, J. C. & Shrubb, M. (2000). Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. – Journal of Applied Ecology 37(5): 771–788. DOI: 10.1046/j.1365-2664.2000.00548.x

Cody, M. L. (1985). An introduction to habitat selection in birds. – In: Cody, M. L. (ed.) Habitat Selection in Birds. – Academic Press, Orlando, FL U.S.A, pp. 3–56.

Cramp, S. (1994). Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic, Vol. 8. – Oxford University Press, Oxford

Denac, D. (2003). Upad populacije in sprememba rabe tal v lovnem habitatu rjavega srakoperja *Lanius collurio* v Šturmovcih (SV Slovenija) [Population decline and land-use changes in hunting habitat of the Red-backed Shrike *Lanius collurio* at Šturmovci (NE Slovenia)]. – Acrocephalus 24(118): 97–102. (in Slovenian with English Summary)

Farkas, R., Horváth, R. & Pásztor, L. (1997). Nesting success of the Red-backed Shrike (*Lanius collurio*) in a cultivated area. – Ornis Hungarica 7: 27–37.

Goławski, A. & Mitrus, C. (2000). Nest site characteristics and breeding success of the Red-backed Shrike (*Lanius collurio*) in agricultural landscape in Eastern Poland. – Ecoscience 21(2): 168–173. DOI: 10.2980/21-2-3653

Golawski, A. (2007). Wielkosc i umieszczenie gniazd gasiorka *Lanius collurio* w krajobrazie rolniczym wschodniej Polski [Size and location of the Red-backed Shrike Lanius collurio nests in the agricultural landscape of eastern Poland]. – Notatki Ornitologiczne 48(4): 273–277. (in Polish with English Summary)

Gregory, R. D., Voříšek, P., Noble, D. G., Van Strien, A., Klvanova, A., Eaton, M., Gmeling Meyling, A. W., Joys, A., Foppen, R. P. B. & Burfield, I. J. (2008). The generation and use of bird population indicators in Europe. – Bird Conservation International 18(Suppl.1): 223–244. DOI: 10.1017/S0959270908000312

Hanane, S. (2012). Do age and type of plantings affect Turtle Dove Streptopelia turtur nest placement in olive-agroecosystems. – Ethology Ecology & Evolutiuon 24(3): 284–293. DOI: 10.1080/03949370.2011.634439

Hanane, S. (2014). Plasticity in nest placement of the Turtle Dove (*Streptopelia turtur*): experimental evidence from Moroccan agroecosystems. – Avian Biology Research 7(2): 65–73. DOI: 10.3184/175815514X13947297062869

Hildén, O. (1965). Habitat selection in birds: a review. – Annales Zoologici Fennici 2(1): 53–75.

Holan, V. (1995). Population density and breeding biology of Red-backed Shrikes in Czechoslovakia. – Proceedings Western Foundation Vertebrate Zoology 6: 76–77.

Horváth, L. (1959). The life history of the Lesser Grey Shrike in Hungary. – Acta Zoologica Hungarica 4(3–4): 319–332.

Horváth, R., Farkas, R. & Yosef, R. (2000). Nesting ecology of the Red-backed Shrike (*Lanius collurio*) in northeastern Hungary. – Ring 22(1): 127–132.

Jakober, H. & Stäuber, W. (1981). Habitatansprüche des Neuntöters *Lanius collurio*. Ein Beitrag zum Schutz einer gefährdeten Art [Habitat claims of the red-backed shrike *Lanius collurio*. A contribution to the protection of an endangered species]. – Ökologie der Vögel 3(2): 223–247. (in German, with English Summary)

Kosiński, Z. (2001). The breeding ecology of the Greenfinch *Carduelis chloris* in urban conditions (study in Krotoszyn, W Poland). – Acta Ornithologica 36(2): 111–121. DOI: 10.3161/068.036.0203

Kotrošan, D., Dervović, I., Topić, G., Drocić, S., Gotovac, M., Šarac, M. & Šarčević, I. (2013). Rezultati prvog popisa sivog svračka (*Lanius minor*) na kraškim poljima Bosne i Hercegovine [Results of first census of Lesser Grey Shrike (*Lanius minor*) in karst poljes of Bosnia and Herzegovina]. – Bilten Mreže posmatrača ptica u Bosni i Hercegovini 9(9): 40–46. (in Croatian)

Krištín, A. (1995). Why the Lesser Grey Shrike (*Lanius minor*) survives in Slovakia: food and habitat preference, breeding biology. –Folia Zoologica 44(4): 325–334.

Krištín, A., Hoi, H., Valera, F. & Hoi, C. (2000). Breeding biology and breeding success of the Lesser Grey Shrike *Lanius minor* in a stable and dense population. – Ibis 142(2): 305–311. DOI: 10.1111/j.1474-919X.2000.tb04870

Kuźniak, S. (1991). Breeding ecology of the Red-backed Shrike *Lanius collurio* in the Wielkopolska region (Western Poland). – Acta Ornithologica 26(2): 67–84. DOI: 83-85192-15-8

Lefranc, N. & Worfolk, T. (1997). Shrikes: A Guide to Shrikes of the World. – Pica Press, Sussex, England

Lislevand, T. (2012). Habitat and nest placement of Red-backed Shrikes *Lanius collurio* breeding in clear-cuts in southern Norway. – Ornis Norvegica 35: 28–36. DOI: 10.15845/on.v35i0.242

Lomáscolo, S. B., Monmany, A. C., Malizia, A. & Martin, T. E. 2010. Flexibility in nest-site choice and nesting success of Turdus rufiventris (Turdidae) in a montane forest in Northwestern Argentina. – The Wilson Journal of Ornithology 122(4): 674–680. DOI: 10.1676/09-167.1

Lovászi, P., Bártol, I. & Moskát, C. (2000). Nestsite selection and breeding success of the Lesser Grey Shrike (*Lanius minor*) in Hungary – Ring 221(1): 157–164.

Marić, D. (2022a). A preliminary assessment of the ornithological importance of karst poljes at Bosansko Grahovo (Western Bosnia and Herzegovina) - results of a large-scale survey in period 1974 to 2020. –The Journal Agriculture and Forestry 68(1): 95–113. DOI: 10.17707/AgricultForest.68.1.05

Marić, D. (2022b). The emigration of the population from the area of Western Bosnia and Herzegovina during the Balkans civil conflict and implications for the Avifauna (Case study Pašića polje at Bosansko Grahovo)–Ecologica Montenegrina 53: 73–91. DOI: 10.37828/em.2022.53.8

Marić, D. (2023). Climate change and implications on the ornithofauna - Case study of the Eurasian Magpie. – The Journal Agriculture and Forestry 69(2) :45–57. DOI: 10.17707/AgricultForest.69.2.04

Marques, P. A. M., Vicente, L. M. & Marquez, R. (2002). Nest placement in the Spanish Sparrow Passer hispaniolensis. – Studies on Sparrows 29(1): 21–30.

Martin, T. E. & Geupel, G. R. (1993). Nest- monitoring plots: Methods for locating nests and monitoring success. – Journal of Field Ornithology 64(4): 507–519.

Moga, C., I., Hartel, T., Öllerer, K. & Szapanyos, Á. (2010). Habitat use by the endangered Lesser Grey Shrike Lanius minor in Central Romania. – Belgian Journal of Zoology 140(2): 223–226.

Nikolić, K. (2021). Od "Ljeta" do "Oluje". Uvod u pad Republike Srpske Krajine 1995. godine [From operation "Summer" to operation "Storm" 1995. Background to the Fall of the Republic of Srpska Krajina]. – Tragovi 4(2): 7–66. DOI: 10.52328./t.4.2.1 (in Serbian, with English Summary)

Nikolov, B. (2000). An investigation of nest building and nests of the Red-backed Shrike (*Lanius collurio*) in Bulgaria. – Ring 22(1): 133–146.

Obratil, S. (1984). Naselja ptica (Aves) u kopnenim biocenozama kraških polja Hercegovine [Communities of birds (Aves) in terrestrial biocenoses of the karst fields of Herzegovina]. – Glasnik zemaljskog muzeja Bosne i Hercegovine (PN) NS 23: 147–184. (in Serbo-Croatian, with English Summary)

Obratil, S. (1987). Naselje ptica u ekosistemima Gatačkog polja i okoline prije izgradnje TE Gacko [Communities of birds in the ecosystems of Gatački polje and its surroundings before the construction of the Gacko TPP]. – Glasnik zemaljskog muzeja Bosne i Hercegovine (PN) NS 25–26: 211–237. (in Serbo-Croatian, with English Summary)

Obratil, S. (2006). Ekološka diferencijacija faune ptica Livanjskog polja (Bosna) [Ecological differentiation of the bird fauna of Livanja polje (Bosnia)]. – Glasnik zemaljskog muzeja Bosne i Hercegovine (PN) NS 32: 45–81. (in Croatian, with English Summary)

Olsson, V. (1995a). The Red-backed Shrike *Lanius collurio* in southeastern Sweden: habitat and territory. – Ornis Svecica 5(1): 31–41.DOI: 10.34080/os.v5.23012

Olsson, V. (1995b). The Red-backed Shrike *Lanius collurio* in southeastern Sweden: breeding biology – Ornis Svecica 5(2–4): 101–110. DOI: 10.34080/os.v5.22999

Pedersen, P. E., Petersen, T. L., Jørgensen, P. S. & Tøttrup, A. P. (2011). The breeding population of Red-backed Shrike *Lanius collurio* in Gribskov, Denmark. – Dansk Ornitologisk Forenings Tidsskrift (DOFT) 105(3): 179–182.

Peterson, R., Mountfort, G. & Hollom, P. A. D. (1968). Die Vogel Europas (achte auflage) [Birds of Europe]. – Verlag Paul Parey, Hamburg und Berlin (in German)

Phalan, B., Onial, M., Balmford, A. & Green, R. E. (2011). Reconciling food production and biodiversity conservation: land sharing and land sparing compared. – Science 333(6047): 1289–1291. DOI: 10.1126/science.1208742

Polak, M. (2012). Habitat preferences of the sympatric Barred Warbler (*Sylvia nisoria*) and the Red-backed Shrike (*Lanius collurio*) breeding in central Poland. – Annales Zoologici Fennici 49(5–6): 355–363. DOI: 10.5735/086.049.0509

Puzović, S., Vučković, V., Stojnić N., Sekulić, G., Radaković, M., Dučić, N., Rudić, B., Ružić, M., Radišić, D., Grubač, B., Šćiban, M. & Raković, M. (2019). Birds of Pešter karst polje. – In: Sackl, P., Ferger, S., Sarajlić, N., Kotrošan, D. & Topić, G. (eds.) Dinaric Karst Poljes-Nature Conservation and Rural Development. – Ornitološko društvo "Naše ptice" Sarajevo pp. 51–85.

Queiroz, C., Beilin, R., Folke, C. & Lindborg, R. (2014). Farmland abandonment: Threat oropportunity for biodiversity conservation? A global review. – Frontiers in Ecology and the Environment 12(5): 288–296. DOI: 10.1890/120348.

Sakhvon, V. & Kövér, L. (2020). Distribution and habitat preferences of the urban Woodpigeon (*Columba palumbus*) in the north-eastern breeding range in Belarus. –Landscape and Urban Planning 201: 1–7. DOI: 10.1016/j.landurbplan.2020.103846

Sfougaris, A. I., Plexida, S. G. & Solomou, A. D. (2014). Assessing the effects of environmental factors on the presence and density of three shrike species in a continental and a coastal area of central Greece. – North-West Journal of Zoology 10(1): 101–109.

Söderström, B. (2001). Seasonal change in Red-backed Shrike *Lanius collurio* territory quality - the role of nest predation. – Ibis 143(1): 561–571. DOI: 10.1111/j.1474-919X.2001.tb04883.x

Sokal, R. & Rohlf, F. J. (1981). Biometry, 2nd ed. – Freeman W.H. and Company Publishers, New York

Sutcliffe, L. M. E., Batáry, P., Kormann, U., Báldi, A., Dicks, L. V., Herzon, I., Kleijn, D., Tryjanowski, P., Apostolova, I., Arlettaz, R., Aviron, S., Balezentiene, L., Fischer, C., Halada, L., Hartel. T., Helm, A., Hristov, J., Jelaska, S. D., Kaligaric, M., Kamp, J., Klimek, S., Koorberg, P., Kostiukova, J., Kovacs-Hostyanszki, A., Kuemmerle, T., Leuschner, C., Lindborg, R., Loos, J., Maccherini, S., Marja, R., Mathe, O., Paulini, I., Proenca, V., Rey-Benayas, J., Sans, F. X., Seifert, C., Stalenga, J., Timaeus, J., Török, P., van Swaay, C., Viik, E. & Tscharntke, T. (2015). Harnessing the biodiversity value of Central and Eastern European farmland. – Diversioti and Distribution 21(6): 722–730. DOI: 10.1111/ddi.12280

Svendsen, J. K., Sell, H., Bøcher, P. K. & Svenning, J-C. (2015). Habitat and nest site preferences of Red-backed Shrike (*Lanius collurio*) in western Denmark. – Ornis Fennica 92(2): 63–75.

Teillard, F, Jiguet, F. & Tichit, M. (2015). The response of farmland bird communities to agricultural intensity as influenced by its spatial aggregation. – PLoS ONE 10(3): e0119674. DOI: 10.1371/journal.pone.0119674

Tryjanowski, P. & Sparks, T. H. (2001). Is the detection of the first arrival date of migrating birds influenced by population size? A case study of the Red-backed Shrike *Lanius collurio*. – International Journal of Biometeorology 45(4): 217–219. DOI: 10.1007/s00484-001-0112-0

Tucker, G. M. & Evans, M. I. (1997). Habitats for Birds in Europe: A Conservation Strategy for the Wider Environment. Birdlife International, Cambridge. Ser 6.

Tucker, G., Heath, M., Tomialojc, L. & Grimmett, R. F. A. (1994). Birds in Europe, the conservation status. Birdlife Conservation Series 3. – BirdLife International, Cambridge DOI: 10.5860/choise 34-0930b

Wilson, R. R. & Cooper, R. J. (1998). Acadian flycatcher nest placement: Does placement influence reproductive success? – The Condor 100(4): 673–679. DOI: 10.2307/1369748

Wirtitsch, M., Hol, H., Valera., F. & Krištín, A. (2001). Habitat composition and use in the Lesser Grey Shrike *Lanius minor*. – Folia Zoologica 50(2): 137–150.

Yosef, R, (2008). Family *Laniidae* (Shrikes). – In: del Hoyo, J., Elliott, A., Christie, D. (eds.) Handbook of the Birds of the World. Vol. 13, Penduline-tits to Shrikes. – Barcelona, Lynx Edicions, pp. 732–773.

Yosef, R. (1994). Evaluation of the global decline in the true shrikes (Family *Laniidae*). – The Auk 111(1): 228–233. DOI: 10.2307/4088532

Zhang, Z. W., Liang, W. & Sheng, G. (1994). Studies on the nest site selection of Daurian Partridge. – Zoological Research 15(4): 37–43.

Žolner, J. (1983). Ku hniezdeniu strakoša obyčajného (*Lanius collurio* L.) na juhovýchode Slovenského krasu [To the nesting of the common magpie (*Lanius collurio* L.) in the southeast of the Slovak Karst]. – Zprávy Moravského ornitologického sdružení 41: 87–110. (in Slovak with English Summary) DOI: 10.1012/675-8-9

Bouayad, F.E., El Idrysy, M., Ouallali, A., El Amrani, M., Courba, S., Hahou, Y., Benhachmi, M.K., Spalevic, V., Kebede F. Briak, H. (2023). Assessing soil erosion dynamics in the Rmel watershed, northern Morocco by using the RUSLE model, GIS, and remote sensing integration. Agriculture and Forestry, 69 (4): 173-194. doi:10.17707/AgricultForest.69.4.11

DOI: 10.17707/AgricultForest.69.4.11

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ASSESSING SOIL EROSION DYNAMICS IN THE RMEL WATERSHED, NORTH-WESTERN MOROCCO BY USING THE RUSLE MODEL, GIS, AND REMOTE SENSING INTEGRATION

SUMMARY

Soil erosion induced by water constitutes a challenge with far-reaching environmental and socioeconomic implications across diverse global regions. This phenomenon detrimentally affects agricultural yield, accelerates dam siltation, and amplifies the susceptibility to flooding. Consequently, a prerequisite for any land development initiative is the meticulous identification and mapping of areas prone to erosion. The Revised Universal Soil Loss Equation (RUSLE) is the predominant method for evaluating soil erosion, encompassing climate erosivity, topography, vegetation cover, soil erodibility, and anti-erosion interventions. This study integrated RUSLE with Geographic Information Systems (GIS) to delineate soil losses within the Rmel watershed in north-western Morocco. The outcomes unveiled an average annual erosion rate of approximately 15.8 tons per hectare, a comparatively modest figure with adjacent regions. Merely 9% of the watershed exhibits vulnerability to soil erosion, surpassing the threshold of 15 tons per hectare annually. These vulnerable areas are predominantly influenced by anthropogenic activities in the basin's central region and adverse climatic conditions downstream. The insights from this research can inform decision-makers in developing strategic action plans and policies for effective soil erosion management in the region. Additionally, the integration of magnetic susceptibility could serve as a complementary tool to enhance the robustness of this analysis.

Keywords: Soil erosion, RUSLE model, GIS, Rmel watershed, Morocco.

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INTRODUCTION

Water erosion, resulting from the degradation of surface soil layers and the displacement of constituent materials (Rodrigues Neto, 2022; Spalevic, 2011, Kavian *et al.* 2018), occurs due to energy release induced by raindrop impact, wind, glaciers, and soil particle transportation (Bhat *et al.* 2019). This widespread phenomenon inflicts significant environmental damage, profoundly affecting ecological and socioeconomic aspects (Moukhchane *et al.* 1998; Spalevic *et al.*, 2013; Saikumar *et al.* 2022; Sabri *et al.* 2022). Globally, erosion poses a serious threat, particularly in Mediterranean countries where factors such as irregular rainfall, elevated temperatures, and a topography characterized by hills and mountains exacerbate soil vulnerability (García-Ruiz *et al.* 2013; Ouallali *et al.* 2020; Salhi *et al.* 2023). A 1977 FAO study revealed that 12.6 million hectares of crops and rangelands in Morocco were at risk of water erosion (El Jazouli *et al.* 2019; Fartas *et al.* 2022). A subsequent 1990 FAO study indicated a worsening scenario, with water erosion affecting 40% of the land area.

Water erosion is the foremost menace to soil degradation in Morocco, with annual soil loss generally exceeding 50 t/ha/y (Salhi *et al.* 2021). The Moroccan mountains present complex erosion challenges, including sheet erosion, gullying, soil movement, bank undermining, solifluction, and mudflows (Roose, 2002). The Rif region experiences a more insidious form of erosion, impacting soil fertility and dam reservoir water volume due to silting (Ouallali *et al.* 2020).

Despite the topographical, lithological, and climatic characteristics of the Oued Rmel watershed, it is not impervious to water erosion phenomena. Integrating remote sensing and Geographic Information Systems (GIS) emerges as crucial tools in interactive decision support and operational planning for risk management operations.

Soil erosion modelling is one of the steps used to plan suitable soil protection measures and detect erosion hotspots (Bezak et *al.* 2021). Various methods exist to determine erosion rates or states, including hydrological modelling methods (Briak *et al.* 2016), geochemical tracers (Guzmán *et al.* 2013), surface geophysical techniques (Ibrahim *et al.* 2020), magnetic susceptibility (Ouallali *et al.* 2023), and empirical modelling (Spalevic *et al.* 2013; Sakuno *et al.* 2020), with the Universal Soil Loss Equation (USLE), its modified version (MUSLE), and its revised version (RUSLE) being the most widely employed (Zhang *et al.* 2009; Gwapedza *et al.* 2018; Djoukbala *et al.* 2019). The optimal model selection depends on the study area's variability and data availability (Stefanidis *et al.* 2022).

The USLE/RUSLE model, grounded in mathematical equations applied in field observations and laboratory analyses, stands among the foremost mathematical models for predicting soil erosion losses (Römkens *et al.* 2015; Ed-daoudy *et al.* 2023). The RUSLE model computes the long-term average annual erosion rate by factoring in rainfall, soil type, topography, vegetation cover, and erosion control practices, allowing for a thorough assessment of erosional impact over time.

This study's primary objective is to determine and map sediment-producing areas in the Rmel watershed. The study also looked into the repercussions of erosion on the water potential of the Rmel dam. The dam, pivotal in providing irrigation and potable water, is also crucial in flood protection for the Tangier-Med port and Ksar Essghir. Through this investigation, we aim to show the complex interaction between erosion dynamics and the essential functions of the Rmel dam in sustaining agricultural, residential, and industrial needs in the region.

MATERIAL AND METHODS

Study area

The Oued Rmel watershed is situated in northwest Morocco, within the province of Ksar Essghir in the Rif region, approximately 44 km northeast of Tangier (Figure 1).

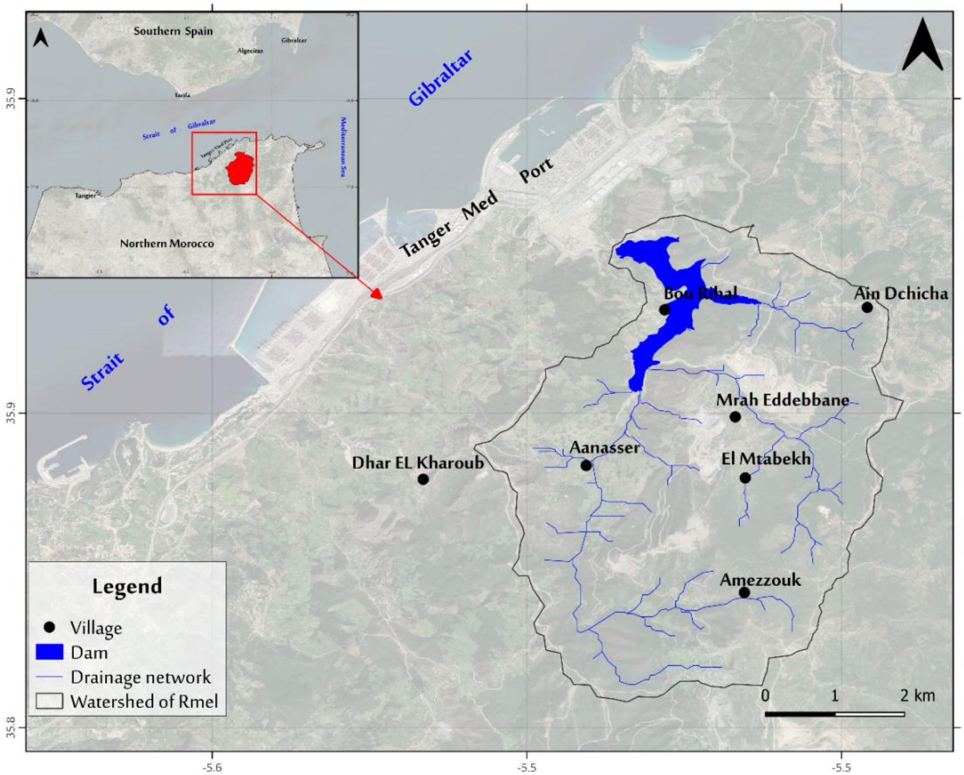

Fig 1: Localization map of the study area

A prominent hydraulic structure in this area is the Rmel dam, constructed in 2008 along the main river. This dam is paramount for hydroelectric power generation, irrigation, water supply, and flood prevention. Boasting a storage capacity of up to 400 million cubic meters, it plays a pivotal role in regional water resource management.

The hydrographic network of the Rmel basin spans a total length of 12.7km and is nourished by rainwater and mountain streams. The primary river within the basin is the Oued Rmel. The climate in the Rmel watershed is categorized as subhumid. Climatic data reveals an average annual rainfall of approximately 400mm, with notable variability from one year to the next, occasionally leading to severe drought conditions. The heaviest rainfall typically occurs in January and February. The region experiences an average temperature of around 17.8°C, with peak temperatures reaching 29.3°C in summer and minimum temperatures averaging 6.3°C in winter. Geologically, the Rmel watershed is characterized by sedimentary deposits, primarily composed of limestone, sandstone, marl, and clay, spanning from the Paleozoic to the Quaternary age. This geological composition contributes to the diverse landscape and environmental dynamics observed in the region.

Model implementation

Various models, differing in complexity and data prerequisites, offer the capability to estimate soil erosion (Karydas *et al.* 2014). These models are versatile in forecasting soil erosion rates across diverse temporal and spatial scales. Notably, the Universal Soil Loss Equation (USLE) model underwent refinement, evolving into the Revised Universal Soil Loss Equation (RUSLE) (Millward & Mersey, 1999).

This updated model facilitates the assessment of the average annual rate of soil loss and allows for the determination of the spatial distribution of an erosion risk map (Mukanov *et al.* 2019). Widely recognized as the preeminent model for estimating soil loss, the RUSLE guides efforts toward soil conservation to mitigate water erosion. The successful application of this novel model hinges on incorporating diverse data types that accurately capture field conditions. We employed the RUSLE model to pursue our research goals, following the outlined protocol depicted in Figure 2.

According to the RUSLE model (Eq. (1)), soil loss (A) is a multiplicative function based on five elements: topography factor (LS), vegetation cover factor (C), soil erodibility (K) (t.h/MJ.mm), rainfall erosivity (R) (MJ.mm/ha.h.year), and erosion control practices factor (P) interrelated according to the equation:

$$A=R*K*LS*C*P \quad \text{Eq. (1)}$$

with:

A: is the average soil erosion per surface unit (t/h/year);
R: is the rainfall and runoff erosivity factor (Mjmm/ha-H-year);
LS: is the slope length (L) and slope steepness (S)factor;
K: is the soil erodibility factor (t-ha-h/ha-MJ-mm);
C: is vegetation cover, management, and culture practices factor;
P: is the conservation practice factor.

We used different data from several sources to evaluate water erosion in the study area. We describe these data in Table 1.

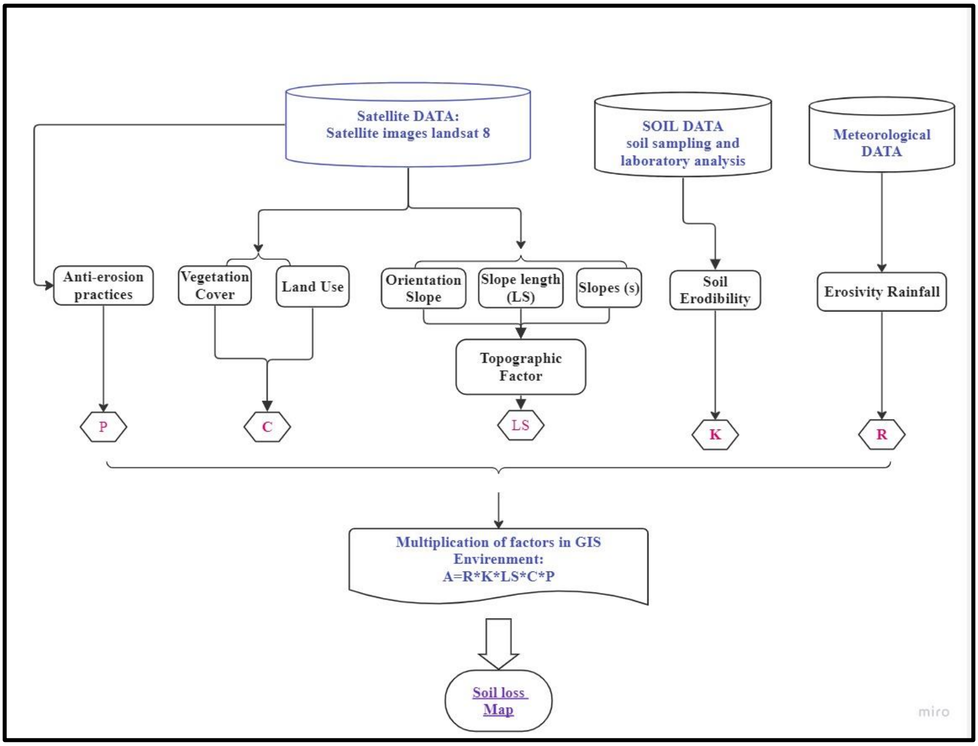

Fig 2: The RUSLE methodology flowchart.

Table 1: Description of datasets used for the RUSLE model.

Datasets	Data source
Rainfall data for the 2010-2022 period recorded at the Tanger Med, Ksar Essghir, Oued Rmel, Alian, Elhorra	ABHL
Data on vegetation cover extracted from Landsat 8 Oli satellite image (no. 201035), downloaded on 05/31/2022	Earth explorer
Topographic data from ASTER 30m digital terrain model (DEM)	Earthdata
Field observations and laboratory analysis of soil samples.	Sample analysis at the laboratory Field observations

To acquire the foundational data essential for constructing maps of water erosion variables in the Oued Rmel watershed, a series of analysis and processing activities are imperative for each component. To estimate soil erosion, a comprehensive dataset of rainfall spanning 12 years (2010-2022) was procured from five stations—Tangier Med, Ksar Essghir, Oued Rmel, Alian, and Elhorra—courtesy of the Loukkos Water Basin Agency. This dataset was instrumental in calculating the rainfall-runoff erosion factor (R factor).

In crafting a rainfall erosion map for the watershed, the rainfall erosion point data underwent interpolation using the Inverse Distance Weighted (IDW) method. This deterministic interpolation method, reliant on the influence of distance for climate station sites, was scrutinized and deemed effective for computing the erosion factor. The resulting R-factor elucidates the spatial distribution of rainfall aggression across the entire area.

Soil type data were employed to assess the soil erodibility factor K. A total of 48 soil samples were meticulously collected in the field and subsequently subjected to physical (particle size analysis) and chemical (organic matter, pH, and electrical conductivity) analyses. These analytical procedures were conducted at the soil chemistry and physics laboratory of the "*Environnement et Conservation des Ressources Naturelles*" research unit at the Rabat Regional Agricultural Research Centre (INRA), contributing to the production of the soil erodibility information layer. The Rmel basin encompasses four primary soil types: vertisol, fluviosol, lithosol, and calcimagnesic soil, as illustrated in Figure3. Each soil type contributes to the basin's diverse and complex soil composition, influencing the local ecosystem and land use characteristics.

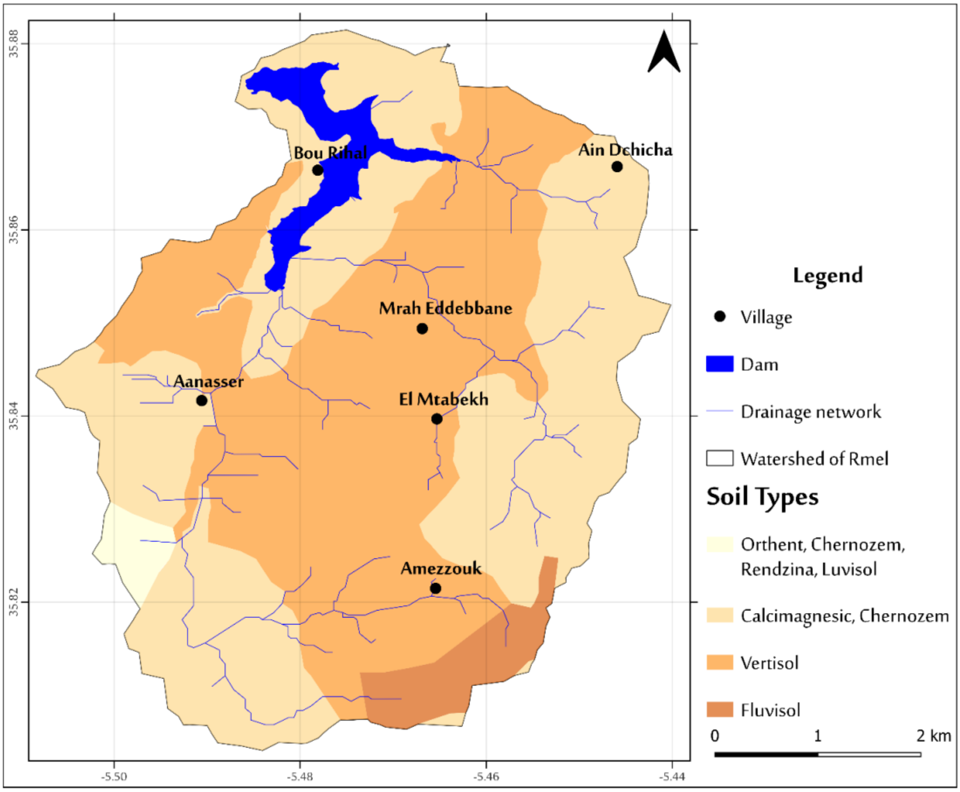

Fig 3: Soil map of the Rmel basin.

On 05/31/2022, a Landsat 8 satellite image was acquired and subsequently subjected to supervised classification using ENVI image processing software.

This process yielded a map illustrating land use unit distribution and the Normalized Difference Vegetation Index (NDVI) calculation. The Rmel watershed exhibits diverse land uses, showcasing rocky outcrops, forests, cultivated land, bare lands, and regions with varying degrees of reforestation, as depicted in Figure 4. This variety in land use contributes to the ecological richness and complexity of the watershed, highlighting the coexistence of natural and anthropogenic elements in the region.

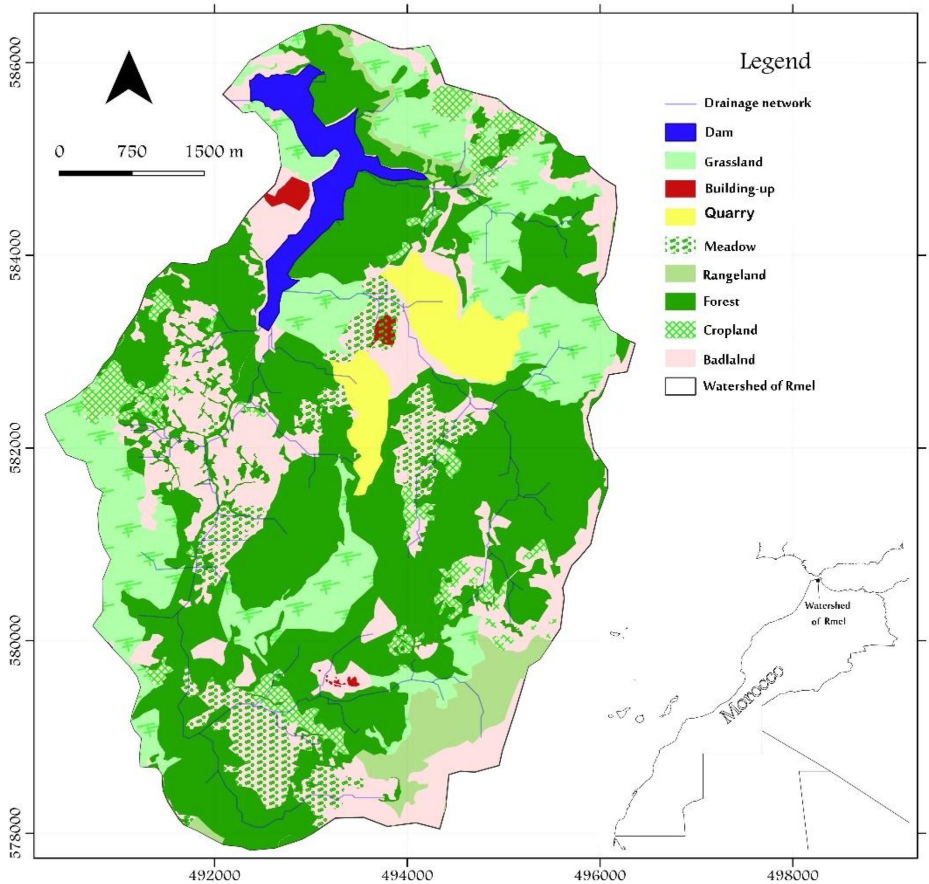

Fig 4: Map of land use in the Rmel basin.

A Digital Terrain Model (DTM) with a spatial resolution of 30m was utilized to augment our understanding of the study area. This DTM facilitated the generation of both a slope map and a flow accumulation map, critical components for the subsequent preparation of the LS factor map in ArcGIS (Rodriguez and Suarez, 2012) (figure 5). The LS factor map, derived from these inputs, serves as a valuable tool in assessing the impact of terrain on soil erosion dynamics, enhancing our capacity to analyse and manage erosion risk within the study region.

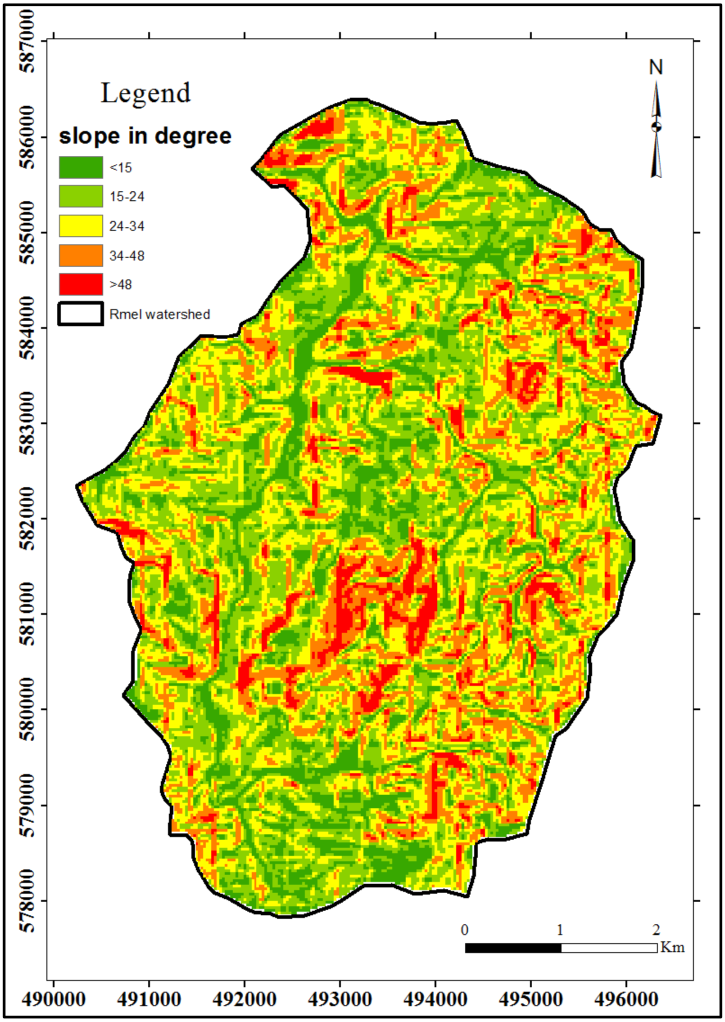

Fig 5. Slope map of the Rmel watershed.

RESULTS AND DISCUSSION

Assessing water erosion factors

The principal objective of this study is to forecast the long-term average annual erosion rate in the Rmel watershed by employing the Revised Universal Soil Loss Equation (RUSLE) model. This predictive model considers various variables, encompassing rainfall patterns, topography, vegetation cover, and agricultural practices.

Rainfall erosivity factor (R)

The kinetic energy of rain plays a substantial role in the removal of solid particles. It is intricately linked to the intensity of the rainfall, influenced by both drop size and velocity (Bartolomé and Teuwen, 2019). The computation of the climatic aggressivity factor, as proposed by Wischmeier and Smith (1978), relies on the understanding of rain kinetic energy and the average rainfall intensity over a 30-minute duration, expressed by the following formula:

$$\mathbf{R=K*EC*I30} \quad \text{Eq. (2)}$$

where:
R: climatic aggressiveness
K: coefficient depending on the unit of measurement
EC: kinetic energy
I30: average intensity of precipitation over 30 min.

The rainfall erosivity factor is expressed in its of M. mm / ha. h. (Rango and Arnoldus, 1987) devised the most widely utilized formula for estimating the R factor, incorporating monthly and annual rainfall. The formulation is articulated as follows:

$$\mathbf{logR= 1.74* log(Pi^2 /P)+1.29} \quad \text{Eq. (3)}$$

where
Pi represents monthly rainfall and P annual rainfall in mm. It is calculated using data from 5 rainfall stations after the results were interpolated over the rest of the basin. The calculation of the erosivity factor R is applied to a series of twelve (12) years of precipitation.

As illustrated in Figure 5, the R-factor values for the Oued Rmel watershed vary between 95.14 and 118.09 M.mm/ha. H (Table 2). The northeastern region exhibits the highest values, whereas the central and southern regions record the lowest values. This spatial pattern indicates increased precipitation aggressiveness from the south to the northeast. This observed gradient is attributed to the altitudinal fluctuations within the watershed, resulting in an elevation-related increment in precipitation levels from the southern to the northeastern regions (Figure 6).

The R-factor values observed in the Oued Rmel watershed ranged from 95.14 to 118.09 M.mm/ha. h, are consistent with findings Ouallali et *al.* (2016) reported for the Oued Arbaa Ayacha watershed in the Western Rif, where values range from 116.633 to 122.615. Comparable patterns are also evident in the Oued Khmis watershed (Western Rif), as reported by Issa *et al.* (2014), with values ranging from 87 to 113. However, the R-factor values in the Oued Rmel watershed surpass those documented in the Oued Leben watershed (central Rif) by Rahhou (1999), where values range from 43 to 87. Dhman *et al.* (1997) identified elevated R-factor values ranging from 215 to 228 in the Telata catchment, and 198.5 to 213 was found in the El Kharoub watershed (Ammari *et al.* 2023).

Table 2: Average annual precipitation (mm) and average R value for 2010-2022.

Station	Average annual precipitation (mm)	R-value
Alian	719.86	112.30
Tanger Med dam	745.69	118.09
Oued Rmel	583.71	96.13
Ksar Essghir	756.78	97.25
El Horra	511.80	95.14

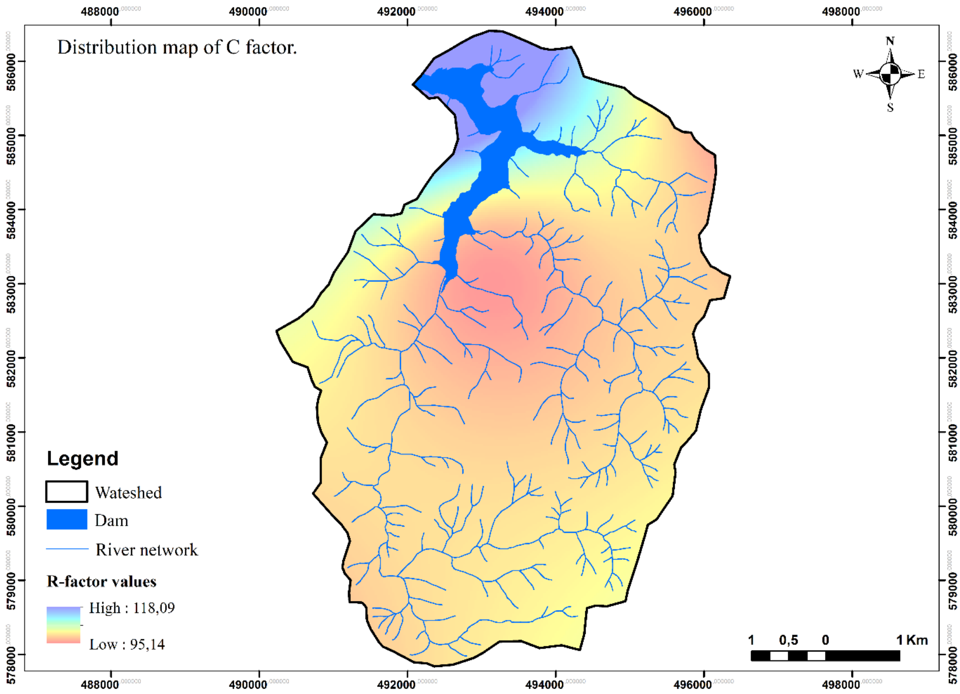

Fig 6: Map of rainfall erosivity factor (R) in the Rmel watershed.

Soil Erodibility Factor (K)

The analysis of pedological properties in the study area facilitated their classification within the Wischmeier and Smith (1978) abacus. This classification was instrumental in estimating the K factor, which is a function of organic matter, structure, texture, and permeability parameters. The K factor holds paramount significance in the Wischmeier and Smith (1978) erosion equation (Eq. 4) as it signifies the degree of soil erodibility, determined through compositional characteristics. The K factor was derived using the following equation (Eq. 4) based on the analysis of soil samples collected within the study area:

$$100K=2.1 * M^{1.4} * 10^{-4}(12-a)+3.25(b-2)+2.5(c-3) \quad \text{Eq. (4)}$$

with, K: Soil erodibility in ha.H/ha.MJ.mm; M: (% Fine sand +% Silt) *(100-% Clay); a: % organic matter; b: soil structure code (1 to 4): 1: very fine; 2: fine; 3: medium and coarse; 4: very coarse c: permeability code (1 to 6): 1 rapid; 2 medium to rapid; 3 moderate; 4 slow to moderate; 5 slow and 6 very slow.

Determining the K factor in the Oued Rmel watershed involved a comprehensive soil sampling study. This study was conducted within the Rmel watershed, encompassing 16 sites where soil samples were collected at three distinct depths. A meticulous methodology, guided by a geological map, guided the sampling process, resulting in 48 carefully preserved and labeled samples. Subsequently, these samples were analyzed at the INRA Rabat laboratory to ensure precision in assessing the soil parameters essential for calculating the K factor.

In the laboratory, the initial processing of samples involved air-drying, followed by grinding and sieving to obtain particle sizes of 2 mm and 0.2 mm, respectively (Figure 7). Subsequently, these prepared samples underwent a comprehensive series of chemical and physical analyses to ascertain their composition and characteristics. This analytical approach was employed to obtain a thorough understanding of the soil properties necessary to determine the K factor in the Oued Rmel watershed.

Fig 7: Soil sample preparation steps
(1: Air drying, 2: Grinding, 3: 2mm sieving, 4: 0.2mm sieving).

Total carbon and organic matter

The soil samples' organic matter content was assessed using the Walkley and Black (1934) method. This method entails the cold oxidation of the organic carbon fraction using 1N potassium dichromate (K2Cr2O7). An initial sulfuric acid (H2SO4) treatment is conducted to mitigate potential chloride interference that might influence results. Following a 2-hour resting period, the surplus dichromate is titrated using 0.5N Mohr's salt solution (Table 3). The organic matter content (%MO) can then be calculated using the following equation:

$$\mathbf{MO\%=\%C*1.724} \quad \text{Eq. (5)}$$

Table 3: Standards for interpreting organic matter (Walkley and Black, 1934).

Class of MO%	**<1.5**	**1.5-3**	**>3**
Interpretation	Low content	Medium content	High content

Particle size analysis

The physical analysis, known as granulometry, evaluates the sample's size and weight proportions of distinct particle categories (Pye and Blott, 2004). This method classifies soil components based on their diameter, providing valuable insights into the particle distribution within the soil sample (Table 4).

Table 4: Granulometric soil texture scale.

Clays	Fine silts	Coarse silts	Fine sands	Coarse sands	Gravel	Pebbles
< 2µm	2-20µm	20-50µm	50-200µm	200µm-2mm	2-20mm	>20mm

Particle size analysis followed the O.R.S.T.O.M (Office of Scientific and Technical Research Outre-Mer, 1937) standard method. This protocol is a well-established procedure designed to ascertain the particle size distribution within a given sample, providing a standardized and reliable approach for characterizing the soil's granulometric composition.

The determined K factor values for the Rmel watershed vary from 0.088t.ha.h/ha.MJ.mm for the most resistant soils to 0.65 t.ha.h/ha.MJ.mm for the most erodible soils. The resulting map illustrates that the soils exhibiting the highest susceptibility to water erosion (K > 0.36 t.ha.MJ.mm/ha) predominantly belong to the class of Vertisols (Table 5).

Vertisols are fertile clay soils known for their high water retention capacity, and they form a distinct band in the central region, roughly oriented from northeast to southwest (Figure 8).

Table 5: Results of the particle size analysis of the samples and value of the K factor

X	Y	%OM(a)	Texture			Texture	K value
			% Sand	% Silt	% Clay		
-5.44618	35,86744	4.3	21.9	34.1	44.0	Clayey	0.17
-5.48867	35,84242	0.1	14.6	40.0	45.4	Clayey	0.25
-5.4896	35,8463	3.4	11.9	52.3	35.8	Clay silt	0.28
-5.48581	35,84874	5.0	18.2	35.6	46.1	Clayey	0.14
-5.48333	35,85681	2.7	8.1	42.9	49.1	Clay sand	0.16
-5.48179	35,86209	3.2	64.5	20.7	14.8	Silt sand	0.62
-5,46294	35,86211	0.6	19.6	34.9	45.6	Clay sand	0.23
-5.46856	35,85608	3.7	21.6	34.6	43.8	Clayey	0.19
-5.47238	35,85372	0.2	27.9	47.0	25.1	Silt	0.58
-5.46551	35,84751	2.0	24.4	56.0	19.6	Silt	0.60
-5,47196	35,84555	0.4	18.1	44.8	37.1	Clay silt	0.35
-5.48735	35,83083	4.1	12.4	47.1	40.5	Clay silt	0.21
-5.47319	35,81139	5.3	9.9	36.0	54.1	Clayey	0.088
-5.47435	35,82016	0.5	54.3	24.4	21.3	Sand	0.65
-5.48655	35,82375	4.9	25.4	30.9	43.7	Clayey	0.16
-5.45976	35,86665	3.5	21.2	35.3	43.5	Clayey	0.19

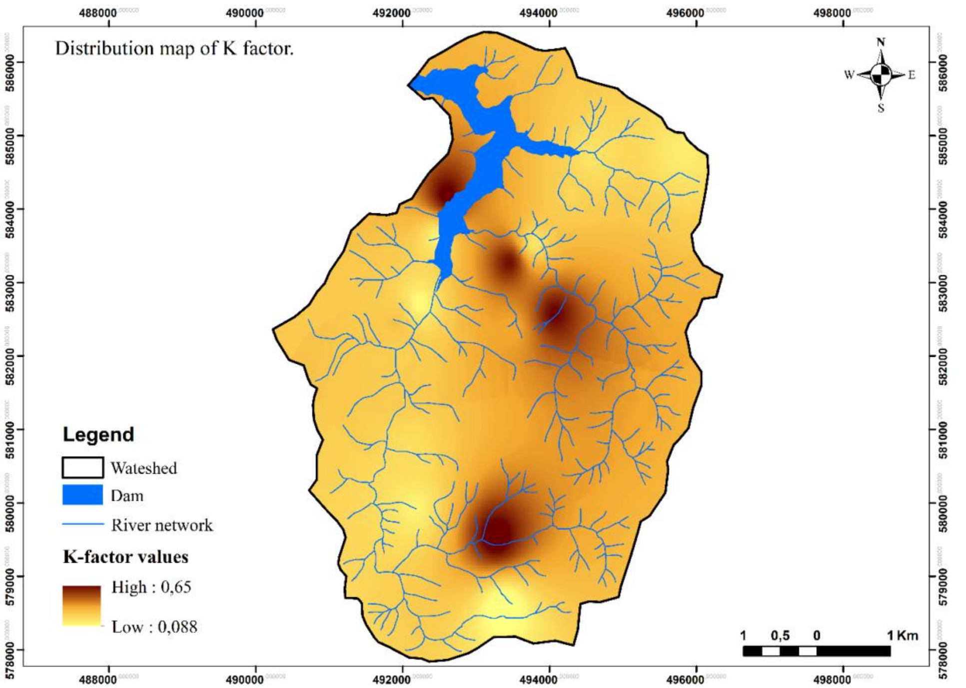

Fig 8: Map of Soil erodibility factor (K) in the Rmel watershed.

Topographic Factor (LS)

The LS factor, commonly known as the "topographic factor," serves as a measure of how the erosion process is influenced by the length of the slope (L) and the steepness of the slope (S) (Anjitha *et al.* 2019). As expressed by the LS factor, these slope-related variables play a pivotal role in shaping the generation and movement of sediment on slopes (Roose 1994). Notably, there is a concurrent increase in soil erosion when development occurs along a slope. The slope's steepness further intensifies this escalation. Moreover, the quantity of vegetation and the size of soil particles influence the dynamics of this soil erosion relationship. The LS factor is mathematically expressed by the following equation (Wischmeier and Smith, 1978):

$$LS = L \div 22.13^{m} \times (0.065 + 0.045S + 0.0065S^{2}) \quad \text{Eq. (6)}$$

where L: slope length, S: slope (%), m: constant dep. on slope value (Tab. 6).

Table 6: The relationship between the parameter m and slope.

Slope (%)	S ≤1	1<S ≤3	3<S ≤5	S>5
m	0.2	0.3	0.4	0.5

The multiplication of flow accumulation and resolution, derived from a digital terrain model, yields slope length (Bircher *et al.* 2019). The ArcGIS Spatial Analyst tool performed slope and flow accumulation computations on the ASTER digital model.

The LS factor calculation reveals a range from 0 to 609.9 (Figure 9) across altitudes spanning 12m to 408m and slopes from 15% to 48%. Elevated LS values predominantly correspond to specific river segments and high-altitude regions characterized by steep slopes. These regions are primarily situated in the central and peripheral zones of the watershed.

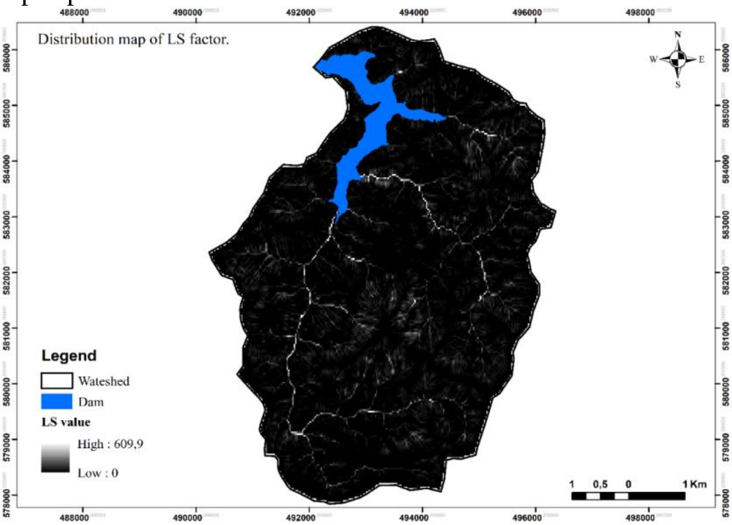

Fig 9. Map of Topographic Factor (LS).

Vegetation cover factor (C)

The influence of vegetation cover is quantified through a dimensionless factor obtained from the normalized vegetation index (NDVI) derived from satellite imagery. This index translates vegetation reflectance into a percentage of vegetation cover (Khunrattanasiri, 2023). However, in response to seasonal canopy variations, our study employs an alternative method, replacing the traditional C-factor with the NDVI vegetation index (Macedo *et al.* 2021).

For our investigation, NDVI is computed from Landsat 8 OLI images at a spatial resolution of 30m. It is utilized to assess the spectral signature, encompassing the near-infrared portion of the electromagnetic spectrum and the red reflection in the upper visible spectrum. The relationship between NDVI and C is expressed by the following equation (Prasannakumar *et al.* 2012):

$$\mathbf{C = \exp(-a\ NDVI/(\beta - NDVI))} \quad \text{Eq. (7)}$$

The C factor spans from 0.1 to 1 (Figure 10). The spatial distribution of these values reveals that 43% of the catchment area exhibits a vegetation cover ranging from low to moderate, with a C factor greater than 0.2. In contrast, 57% of the area seems adequately protected, featuring C values below 0.2, indicating a high level of preservation. These areas encompass virtually the entire basin.

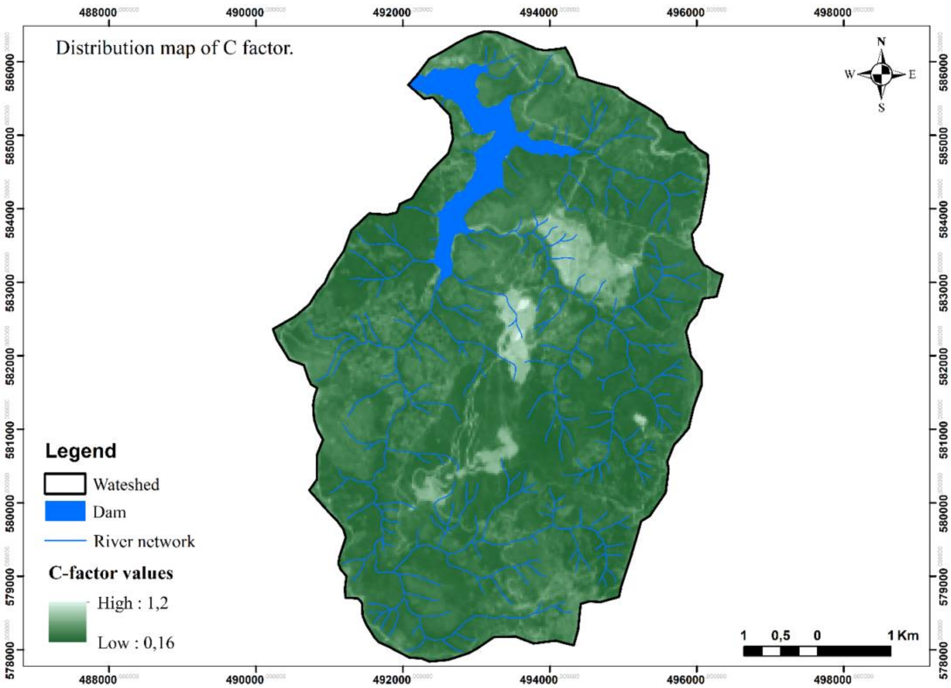

Fig 10. Distribution map of C factor in the watershed

Pratique de gestion des terres factor (P)

The soil conservation practice factor, also called the support practice, indicates the proportion of soil loss assigned to each cultivation method based on its ability to conserve soil from erosion (Panagos *et al.* 2015). This includes contour plowing, ridging, slope terracing, and alternating strip crops. The P factor evaluates the impact of conservation tillage on reducing soil loss (Araya *et al.* 2011). If adequately maintained, erosion control measures influence water flow and land topography, reducing soil loss. The P factor is assigned a value between 0 and 1, where 1 signifies the absence of conservation features.

This metric facilitates the assessment of soil losses in watersheds by considering the effectiveness of existing erosion control techniques. Each measure has a protective degree, with its coefficient determined by how well it minimizes runoff and mitigates erosion causes (Benzougagh *et al.* 2020). Cultivation practices such as contour farming, alternating strips or terraces, reforestation on terraces, and ridging are identified as highly effective soil conservation strategies (Zettam *et al.* 2022). These strategies are evaluated based on the soil loss ratio with specific support on agricultural land compared to the corresponding loss with parallel slope plowing (Zouagui *et al.* 2018).

It is essential to highlight that in our study, areas with low to moderate slopes exhibit the lowest and average P-factor values. Specifically, the P factor ranges between 0.2 and 0.5 for areas with low slopes, while it varies between 0.6 and 0.9 for areas with steep slopes (Figure 11).

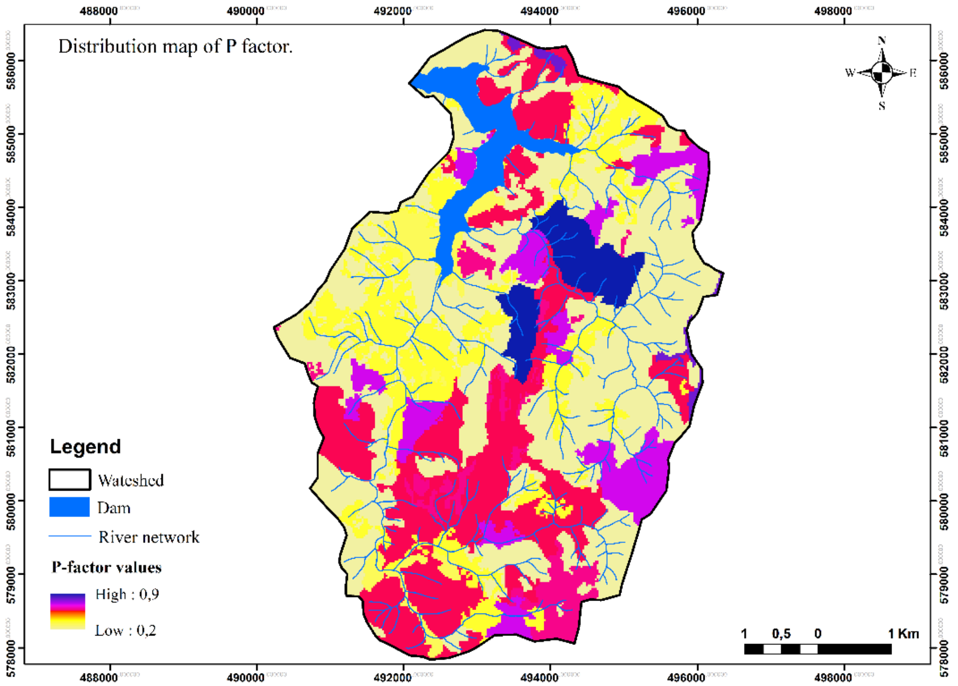

Fig 11: Map of the anti-erosion factor (P) in the watershed Rmel

Erosion rate estimation

The erosion rate, as determined by the RUSLE model, results from the interplay of various factors, with climatic aggressiveness (R), soil erodibility (K), and the combined impact of slope degree and slope length (LS), vegetation cover (C), and erosion control practices (P) being the most influential. Calculating the erosion rate provides insights into the distribution of erosion risk attributable solely to natural factors.

The results indicate a range of soil losses in the region, spanning from less than 7 to over 45 t/ha/year, with an average of approximately 16 t/ha/year. Approximately 60% of the basin area falls within the loss class below 7 t/ha/year, signifying relatively low losses. About 30% of the area is categorized as having moderate losses, falling between 7 and 20 t/ha/year. Areas experiencing high water erosion, exceeding 20 t/ha/year, constitute only 10% of the total Rmel watershed area. These vulnerable zones to water erosion are predominantly situated in the central and north-eastern portions of the watershed (Figure 12).

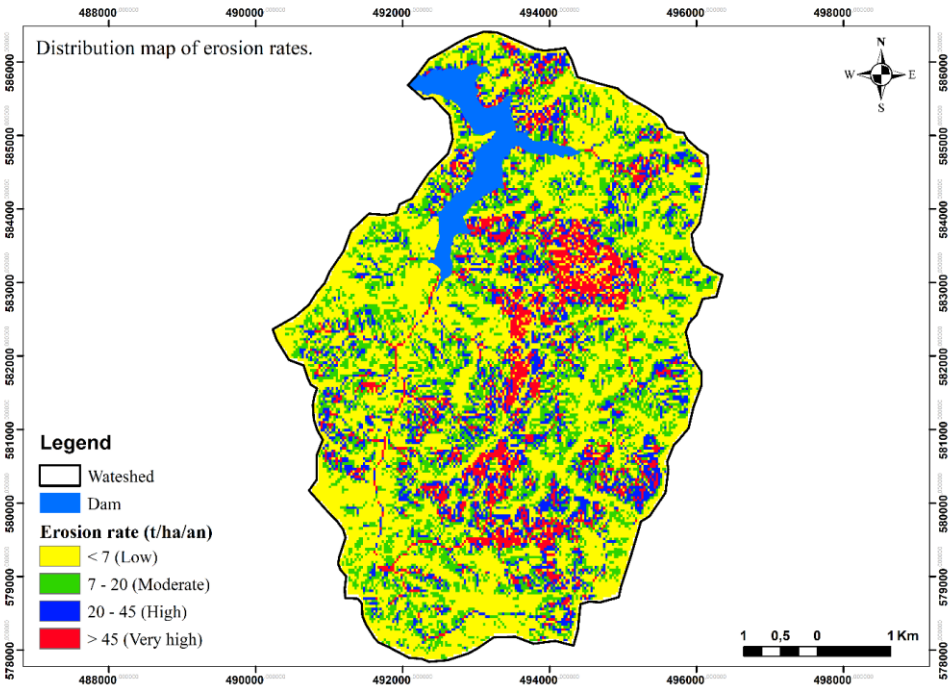

Fig 12: Resulting map of soil losses in t/ha/yr

The areas with a high potential for erosion are typically associated with soils in the central basin regions characterized by steep slopes and intense human activity, particularly quarrying. In the northeastern part, precipitation (climatic aggressiveness) emerges as the primary factor driving water erosion despite relatively abundant vegetation.

On the Rif scale, the Rmel watershed stands out for having the best protection against water erosion. Specifically, the Sania and Arbaa Ayacha watersheds in the western Rif exhibit an average annual loss of approximately 47.18 t/ha (Tahiri *et al.* 2015) and 25.8 t/ha (Ouallali *et al.* 2016), respectively. Comparable values were observed in the eastern Rif, with the Oued Sahla basin recording 22 t/ha/yr and the Oued Boussouabl basin registering 55 t/ha/yr (Sadiki *et al.* 2009).

CONCLUSION

The RUSLE approach for assessing soil water erosion indicates that the Rmel watershed faces a relatively low erosion risk compared to other regions in the Rif. Areas with susceptible soils encompass only 9% of the basin area, exhibiting an average annual erosion rate exceeding 15 t/ha. These vulnerable zones, predominantly situated in the central and downstream parts of the basin, show that human activities and climatic aggressiveness are the primary contributors to soil erosion. On the other hand, areas with low erosion potential, as inferred from land loss estimates, are mainly characterized by cultivated land featuring a topography dominated by low slope classes and experiencing low rainfall erosivity.

The optimized RUSLE equation developed in this analysis could be a valuable resource for future regional land-use planning and management activities; however, caution is advised in its application. Furthermore, it provides a framework for potential soil erosion studies in the study area and other similarly conditioned regions. To calculate the rate of soil loss in the watershed, alternative techniques such as radioactive markers like Caesium 137 (137Cs), PAP/CAR, the SWAT model (Soils Water Assessment Tools), the IntErO model (Spalevic, 2011), River Basins model (Spalevic, 1999) and the SAM model (Spectral Angel Mapper) could also be considered.

REFERENCES

Ammari, Z., El Mostafa Mili, A. E., El Hafyani, M., Essahlaoui, N., Boufala, M. H., Ouallali, A., Berrad, F., & Aassoumi, H. (2023). Mapping and predicting of water erosion using RUSLE in the mediterranean context: case of El Kharroub watershed (western rif, northern morocco). ARPN Journal of Engineering and Applied Sciences, 18,6.

Anjitha Krishna, P. R., Lalitha, R., Shanmugasundaram, K., & Nagarajan, M. (2019). Assessment of topographical factor (LS-factor) estimation procedures in a gently sloping terrain. Journal of the Indian Society of Remote Sensing, 47, 1031-1039.

Araya, T., Cornelis, W. M., Nyssen, J., Govaerts, B., Bauer, H., Gebreegziabher, T., & Deckers, J. (2011). Effects of conservation agriculture on runoff, soil loss and crop yield under rainfed conditions in Tigray, Northern Ethiopia. Soil Use and Management, 27(3), 404-414.

Bartolomé, L., & Teuwen, J. (2019). Prospective challenges in the experimentation of the rain erosion on the leading edge of wind turbine blades. Wind Energy, 22(1), 140-151.

Benzougagh, B., Meshram, S. G., Baamar, B., Dridri, A., Boudad, L., Sadkaoui, D., & Mimich, K. (2020). Relationship between landslide and morpho-structural analysis: a case study in Northeast of Morocco. Applied Water Science, 10(7), 1-10.

Bezak, N,; Mikoš, M.; Borrelli, P.; Alewell, C.; Alvarez, P.; Alexandre Ayach Anache, J.; Baartman, J.; Ballabio, C.; Biddoccu, M.; Cerdà, A.; Chalise, D.; Chen, S.; Chen, W.; De Girolamo, A.; Desta Gessesse, G.; Deumlich, D.; Diodato, N.; Efthimiou, N.; Erpul, G.; Fiener, P.; Freppaz, M.; Gentile, F.; Gericke, A.; Haregeweyn, N.; Hu, B.; Jeanneau, A.; Kaffas, K.; Kiani-Harchegani, M.; Lizaga Villuendas, I.; Li, C.; Lombardo, L.; López-Vicente, M.; Esteban Lucas-Borja, M.; Maerker, M.; Miao, C.; Modugno, S.; Möller, M.; Naipal, V.; Nearing, N.; Owusu, S.; Panday, D.; Patault, E.; Valeriu Patriche, C.; Poggio, L.; Portes, R.; Quijano, L.; Reza Rahdari, M.; Renima, M.; Francesco Ricci, G.; Rodrigo-Comino, J.; Saia, S.; Nazari Samani, A.; Schillaci, C.; Syrris, V.; Soo Kim, H.; Noses Spinola, D.; Tarso Oliveira, P.; Teng, H.; Thapa, R.; Vantas, K.; Vieira, D.; Yang, J.; Yin, S.; Antonio Zema, D.; Zhao, G. & Panagos, P. (2021). Soil erosion modelling: A bibliometric analysis. Environmental Research, 197, 111087

Bhat, S. A., Dar, M. U. D., & Meena, R. S. (2019). Soil erosion and management strategies. Sustainable management of soil and environment, 73-122.

Bircher, P., Liniger, H. P., & Prasuhn, V. (2019). Comparing different multiple flow algorithms to calculate RUSLE factors of slope length (L) and slope steepness (S) in Switzerland. Geomorphology, 346, 106850.

Briak, H., Moussadek, R., Aboumaria, K., & Mrabet, R. (2016). Assessing sediment yield in Kalaya gauged watershed (Northern Morocco) using GIS and SWAT model. International Soil and Water Conservation Research, 4(3), 177-185.

Djoukbala, O., Hasbaia, M., Benselama, O., & Mazour, M. (2019). Comparison of the erosion prediction models from USLE, MUSLE and RUSLE in a Mediterranean watershed, case of Wadi Gazouana (NW of Algeria). Modeling Earth Systems and Environment, 5(2), 725-743.

Ed-daoudy, L., Lahmam, N., Benmansour, M., Afilal, H., & Damnati, B. (2023). Hydric erosion rates in Raouz watershed, Morocco: RUSLE, GIS, and remote sensing. Remote Sensing Applications: Society and Environment, 32, 101056.

El Jazouli, A., Barakat, A., Khellouk, R., Rais, J., & El Baghdadi, M. (2019). Remote sensing and GIS techniques for prediction of land use land cover change effects on soil erosion in the high basin of the Oum Er Rbia River (Morocco). Remote Sensing Applications: Society and Environment, 13, 361-374.

Fartas, N., El Fellah, B., Mastere, M., Benzougagh, B., & El Brahimi, M. (2022). Potential Soil Erosion Modeled with RUSLE Approach and Geospatial Techniques (GIS Tools and Remote Sensing) in Oued Joumouaa Watershed (Western Prerif-Morocco). The Iraqi Geological Journal, 47-61.

García-Ruiz, J. M., Nadal-Romero, E., Lana-Renault, N., & Beguería, S. (2013). Erosion in Mediterranean landscapes: changes and future challenges. Geomorphology, 198, 20-36.

Guzmán, G., Quinton, J. N., Nearing, M. A., Mabit, L., & Gómez, J. A. (2013). Sediment tracers in water erosion studies: current approaches and challenges. Journal of Soils and Sediments, 13, 816-833.

Gwapedza, D., Hughes, D. A., & Slaughter, A. R. (2018). Spatial scale dependency issues in the application of the Modified Universal Soil Loss Equation (MUSLE). Hydrological Sciences Journal, 63(13-14), 1890-1900.

Ibrahim, D., Ahmed, B., Habiba, A., Abdessalam, O., & Youssef, A. B. (2020). Contribution of geophysics to the study of Barite mineralization in the Paleozoic formations of Asdaf Tinejdad (Eastern Anti Atlas Morocco). Economic and Environmental Geology, 53(3), 259-269.

Issa, L. K., Raissouni, A., Moussadek, R., El ArrimIssa, A., Khali, L., Raissouni, A., ... & El Arrim, A. (2014). Mapping and assessment of water erosion in the Khmiss Watershed (North Western Rif, Morocco). Current Advances in Environmental Science, 2(4), 119-130.

Karydas, C. G., Panagos, P., & Gitas, I. Z. (2014). A classification of water erosion models according to their geospatial characteristics. International Journal of Digital Earth, 7(3), 229-250.

Kavian, A.; Gholami, L.; Mohammadi, M.; Spalevic, V.; & Falah Soraki, M. (2018). Impact of Wheat Residue on Soil Erosion Processes. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46(2), 553-562.

Khunrattanasiri, W. (2023). Application of Remote Sensing Vegetation Indices for Forest Cover Assessments. In Concepts and Applications of Remote Sensing in Forestry (pp. 153-166). Singapore: Springer Nature Singapore.

Macedo, P. M. S., Oliveira, P. T. S., Antunes, M. A. H., Durigon, V. L., Fidalgo, E. C. C., & de Carvalho, D. F. (2021). New approach for obtaining the C-factor of RUSLE considering the seasonal effect of rainfalls on vegetation cover. International soil and water conservation research, 9(2), 207-216.

Millward, A. A., & Mersey, J. E. (1999). Adapting the RUSLE to model soil erosion potential in a mountainous tropical watershed. Catena, 38(2), 109-129.

Moukhchane, M., Bouhiassa, S., & Chalouan, A. (1998). Approche cartographique et magnétique pour l'identification des sources de sédiments: cas du bassin versant Nakhla (Rif, Maroc). Science et changements planétaires/Sécheresse, 9(3), 227-232.

Mukanov, Y., Chen, Y., Baisholanov, S., Amanambu, A. C., Issanova, G., Abenova, A., ... & Abayev, N. (2019). Estimation of annual average soil loss using the Revised Universal Soil Loss Equation (RUSLE) integrated in a Geographical Information System (GIS) of the Esil River basin (ERB), Kazakhstan. Acta Geophysica, 67, 921-938.

Ouallali, A., Aassoumi, H., Moukhchane, M., Moumou, A., Houssni, M., Spalevic, V., & Keesstra, S. (2020). Sediment mobilization study on Cretaceous, Tertiary and Quaternary lithological formations of an external Rif catchment, Morocco. Hydrological Sciences Journal, 65(9), 1568-1582.

Ouallali, A., Briak, H., Aassoumi, H., Beroho, M., Bouhsane, N., & Moukhchane, M. (2020). Hydrological foretelling uncertainty evaluation of water balance components and sediments yield using a multi-variable optimization approach in an external Rif's catchment. Morocco. Alexandria Engineering Journal, 59(2), 775-789.

Ouallali, A., Moukhchane, M., Aassoumi, H., Berrad, F., Ibrahim, D., (2016). Evaluation and mapping of water erosion rates in the watershed of the Arbaa Ayacha river (western Rif, northern Morocco). Bull. Inst. Sci. Sect. Sci. Terre 38, 65–79.

Ouallali, A.; Bouhsane, N.; Bouhlassa, S.; Moukhchane, M.; Ayoubi, S.; Aassoumi, H. Rapid magnetic susceptibility measurement as a tracer to assess the erosion-deposition process using tillage homogenization and simple proportional models: A case study in northern of Morocco. International Journal of Sediment Research. (2023), 38, 739–753.

Panagos, P., Borrelli, P., Meusburger, K., Van Der Zanden, E. H., Poesen, J., & Alewell, C. (2015). Modelling the effect of support practices (P-factor) on the reduction of soil erosion by water at European scale. Environmental science & policy, 51, 23-34.

Prasannakumar, V., Vijith, H., Abinod, S., & Geetha, N. J. G. F. (2012). Estimation of soil erosion risk within a small mountainous sub-watershed in Kerala, India, using Revised Universal Soil Loss Equation (RUSLE) and geo-information technology. Geoscience frontiers, 3(2), 209-215.

Pye, K., & Blott, S. J. (2004). Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry. Forensic Science International, 144(1), 19-27.

Rahhou, M. (1999). L’érosion dans le Prérif central, zone interfluviale Leben-Sebou-Ouergha, un prolongement de l’évolution naturel, une production sociale. State thesis, Mohammed V University of Rabat, Morocco.

Rango, A., & Arnoldus, H. M. J. (1987). Watershed management. Food and Agriculture Organization Technical Papers. Rome. FAO, 36.

Rodrigues Neto, M.R.; Musselli, D.G.; Lense, G.H.E.; Servidoni, L.E.; Stefanidis, S.; Spalevic, V.; Mincato, R.L. (2022). Soil loss modelling by the IntErO model - Erosion Potential Method in the Machado River Watershed, Minas Gerais, Brazil. Agriculture and Forestry, 68 (2): 7-21.

Rodriguez, J. L. G., & Suarez, M. C. G. (2012). Methodology for estimating the topographic factor LS of RUSLE3D and USPED using GIS. Geomorphology, 175, 98-106.

Römkens, M. J., Wells, R. R., Wang, B., Zheng, F., & Hickey, C. J. (2015). Soil Erosion on Upland Areas by Rainfall and Overland Flow. Advances in Water Resources Engineering, 361-405.

Roose, E. (1994). Introduction à la gestion conservatoire de l'eau, de la biomasse et de la fertilité des sols (GCES) (Vol. 70). Organisation des Nations Unies pour l'alimentation et l'agriculture.

Roose, E. (2002). Traditional strategies for soil and water conservation in Mediterranean areas. Rubio, Josè Luis.

Sabri, E., Spalevic, V., Boukdir, A., Karaoui, I., Ouallali, A., Mincato, R. L., & Sestras, P. (2022). Estimation of soil losses and reservoir sedimentation: a case study in Tillouguite sub-basin (high Atlas-Morocco). Agriculture & Forestry/Poljoprivreda i Sumarstvo, 68(2).

Sadiki, A., Faleh, A., Zêzere, J. L., & Mastass, H. (2009). Quantification de l’Erosion en Nappes dans le Bassin Versant de l’Oued Sahla-Rif Central Maroc. Cahiers géographiques, 6, 59-70.

Saikumar, G., Pandey, M., & Dikshit, P. K. S. (2022). Natural River Hazards: Their Impacts and Mitigation Techniques. In River Dynamics and Flood Hazards: Studies on Risk and Mitigation (pp. 3-16). Singapore: Springer Nature Singapore.

Sakuno, N. R. R., Guiçardi, A. C. F., Spalevic, V., Avanzi, J. C., Silva, M. L. N., & Mincato, R. L. (2020). Adaptation and application of the erosion potential method for tropical soils. Revista Ciência Agronômica, 51.

Salhi, A., Benabdelouahab, T., Hasnaoui, Y. E., El Moussaoui, M., El Morabit, A., Himi, M., ... & Martin-Vide, J. (2021). Soil erosion assessment and farmers' perception in south Mediterranean basins: a Moroccan case study. In Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (2nd Edition) Proceedings of 2nd Euro-Mediterranean Conference for Environmental Integration (EMCEI-2), Tunisia 2019 (pp. 2019-2024). Springer International Publishing.

Salhi, A., El Hasnaoui, Y., Pérez Cutillas, P., & Heggy, E. (2023). Soil erosion and hydroclimatic hazards in major African port cities: the case study of Tangier. Scientific Reports, 13(1), 13158.

Spalevic, V. (1999). Application of computer-graphic methods in the studies of draining out and intensities of ground erosion in the Berane valley. Master thesis, Faculty of Agriculture of the University of Belgrade, Serbia, 1999: 135p.

Spalevic, V. (2011). Impact of land use on runoff and soil erosion in Polimlje. Doctoralthesis, University of Belgrade, Faculty of Agriculture, Serbia, p 1-260.

Spalevic, V., Djurovic, N., Mijovic, S., Vukelic-Sutoska, M., & Curovic, M. (2013). Soil erosion intensity and runoff on the Djuricka River Basin (North of Montenegro). Malaysian Journal of Soil Science, 17(1), 49-68.

Stefanidis, S., Alexandridis, V., Spalevic, V., Mincato, R.L. (2022). Wildfire Effects on Soil Erosion Dynamics: The Case of 2021 Megafires in Greece. Agriculture and Forestry, 68 (2): 49-63. doi:10.17707/AgricultForest.68.2.04

Tahiri, M., Tabyaoui, H., Tahiri, A., El Hadi, H., El Hammichi, F., & Achab, M. (2015). Modelling soil erosion and sedimentation in the Oued Haricha sub-basin (Tahaddart watershed, Western Rif, Morocco): risk assessment. Journal of Geoscience and Environment Protection, 4(1), 107-119.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil science, 37(1), 29-38.

Wischmeier, W. H., & Smith, D. D. (1978). Predicting rainfall erosion losses: a guide to conservation planning (No. 537). Department of Agriculture, Science and Education Administration.

Zettam, A., Briak, H., Kebede, F., Ouallali, A., Hallouz, F., & Taleb, A. (2022). Efficiencies of best management practices in reducing nitrate pollution of the Sebdou River, a semi-arid Mediterranean agricultural catchment (North Africa). River Research and Applications, 38(3), 613-624.

Zhang, Y., Degroote, J., Wolter, C., & Sugumaran, R. (2009). Integration of modified universal soil loss equation (MUSLE) into a GIS framework to assess soil erosion risk. Land Degradation & Development, 20(1), 84-91.

Zouagui, A., Sabir, M., Naimi, M., Chikhaoui, M., & Benmansour, M. (2018). Modélisation du risque d'érosion hydrique par l'équation universelle des pertes en terre dans le Rif Occidental: Cas du bassin versant de Moulay Bouchta (Maroc). European Scientific Journal, 14(3), 1857-788.

Mahmoud, A.A., Astatkie, T., Hikal, W.M., Said-Al Ahl, H.A.H. (2023): Effects of Sodium Chloride stress on growth, development characters and yield of Nigella sativa during vegetative, flowering, and fruiting stages. Agriculture and Forestry, 69 (4): 195-207. doi:10.17707/AgricultForest.69.4.12

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EFFECTS OF SODIUM CHLORIDE STRESS ON GROWTH, DEVELOPMENT CHARACTERS AND YIELD OF NIGELLA SATIVA DURING VEGETATIVE, FLOWERING, AND FRUITING STAGES

SUMMARY

Globally, black cumin (*Nigella sativa* L.) plays a crucial role in human health by being a traditional medicine for treating diseases, and an ingredient for herbal drug formulations and several culinary applications. To maximize its usage, it is important to understand how its growth is affected by stresses. Therefore, an experiment was conducted to determine the effect of saline water irrigation on survival, growth, and yield characteristics of *Nigella sativa*. The plants were treated with four (control=0, 1000, 2000, and 3000 ppm) concentrations of Sodium chloride (NaCl) and its important characteristics (survival, plant height, number of branches/plant, fresh and dry stem weights, fresh and dry leaf weights, total fresh and dry weights, number of umbels/plant, fresh and dry umbels weights, number of capsules/plants, dry capsule weight, dry straw weight, seed weight) were measured during the three growth stages (vegetative, flowering, and fruiting). The results revealed that survival of the plant, its growth, and its productivity significantly decreases with increasing concentration of NaCl at the vegetative and flowering stages. This also caused substantial reduction in the seed yield and yield components at the fruiting stage.

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Our results showed that *Nigella sativa* is sensitive to saline water, and there is a need for irrigating it with minimal salt concentration to reduce the severity of damage in its growth and yield.

Keywords: *Nigella sativa*, sodium chloride, saline irrigation, survival, yield

INTRODUCTION

Traditional herbal medicines, which are naturally occurring plant-based substances used to treat illness, are getting significant attention in therapeutic and nutritional applications (Willcox and Bodker, 2004). More attention for studies on traditional herbal medicine is needed to discover promising medicinal herbs and novel chemical compounds (Tilburt and Kaptchuk, 2008). This is particularly important in Africa because 80% of African populations use some form of traditional herbal medicine (WHO, 2002). There are lots of studies that showed the effectiveness of traditional medicines to treat various diseases such as gastrointestinal diseases, headache and migraine, fertility, diabetes, hyperlipidemia, renal injury, stress and depression, pain, respiratory diseases, neurological disorders, liver disorders, and cancers (Kooti *et al.*, 2016).

One of the most important plants that has been used since ancient times and has a prominent role in folk medicine is black cumin (*Nigella sativa* L.; *Ranunculaceae* family) plant, and it still receives great interest in modern research in the food, cosmetics, and pharmaceutical fields. *Nigella sativa* seed is naturally grown in North Africa, southern Europe, the Middle East, and Southwest Asia; and is widely cultivated for numerous industrial and medicinal purposes (Niu *et al.*, 2020; Shahbazi *et al.*, 2022). *Nigella sativa* seeds stimulate great interest in the food, cosmetics, and pharmaceutical fields. Studies have shown several pharmacological properties of *Nigella sativa* including antibacterial activity, antifungal activity, antimalarial activity, antiviral activity, anti-schistosomal activity, antioxidant activity, anti-inflammatory, anti-hyperlipidemic, anti-cancer activity, anti-diabetic activity, cardiovascular protective activity, gastro-protective, nephro-protective, hepato-protective, neuro-protective, immuno-protective, reproductive system; and healing activities for rheumatoid arthritis, asthma, digestive diseases, anti-hypertensive and wound (Kooti *et al*., 2016; Oyweri *et al.*, 2019; Khalil *et al.*, 2021; Mukhtar *et al.*, 2021). It also has effects on reproductive, digestive, immune, and central nervous systems; and anticonvulsant and analgesic activities (Kooti *et al*., 2016). *Nigella sativa* seeds are also employed as preservatives and spices to flavor foods, such as bakery products, cheese, pickles, and a variety of traditional dishes (Salehi *et al.*, 2021; Benazzouz-Smail *et al.*, 2023). *Nigella sativa* seeds are also a substantial source of nutritionally vital constituents, such as fixed oils (32.2–41%), as well as to the fact that seeds are rich in polyunsaturated fatty acids, proteins (13.8–22%), carbohydrates (17%), ashes (4.5–7.5%), dietary fibers (8–16.4%), and vitamins, such as tocopherol and niacin (Kabir *et al*., 2019; Shahbazi *et al*., 2022; Benazzouz-Smail *et al*., 2023). The mentioned biological properties are related to their richness in several phytochemicals, mainly phenolic compounds, terpenes, essential oils, saponins, and alkaloids (Benazzouz-Smail *et al.,* 2023). As a result of the multiple uses of *Nigella sativa* and its therapeutic benefits, there is a need and interest to study environmental conditions and to identify factors affecting its cultivation and optimal productivity, especially under conditions of salt stress.

To maintain good plant production, there is a need to identify salt-tolerant plants. In studies that aim to achieve this, plants are typically exposed to high levels of salinity to understand the various physiological and biochemical changes, the modifications that occur in the structure, and the function of cell membranes when exposed to salt stress and to enhance salt tolerance of crops (Arzani, 2008; Taarit *et al*., 2010). Since soil salinity is a global challenge due to the expansion of affected land areas, there are more and more studies that explore the different responses of plants to salinity.

Salinity is one of the main factors limiting plant growth and agricultural production in general (Kheloufi and Mansouri, 2019). Many studies focus on the effects of sodium chloride on medicinal and aromatic plants' anatomical, physiological, molecular, chemical, and protein attributes (Šutković *et al.,* 2011). In general, about three-quarters of the total soluble salt commonly used in irrigation water is sodium chloride. Plants vary greatly in their tolerance to saline water; however, the presence of stunted growth is a sign of salinity stress. As salt level in the soil increases to more toxic level, the leaves start to burn and fall off from the plant, which leads to the death of the plant. In other cases, the leaves may appear yellow, or the crop may show signs of wilting, even though the soil appears adequately moist (Flowers *et al*., 2015; Geilfus, 2018; Hegazy *et al*., 2019; Sany *et al.,* 2020).

The negative effects of salinity include causing an imbalance in cellular ions that leads to osmotic stress that makes water absorption difficult (Meloni *et al*., 2001). It also reduces the absorption of nutrients due to the ability of sodium to compete with the basic cations required for cell function (Tester and Davenport, 2003). Several studies reported the negative effects of salinity on the productivity of various aromatic and medicinal plants, such as coriander (Said-Al Ahl *et al*., 2014), black cumin (Bourgou *et al*., 2010), sweet majorana (Baâtour *et al.*, 2013), oregano (Said-Al Ahl and Hussein, 2010), basil (Tarchoune *et al.*, 2013), dill (Said-Al Ahl *et al.*, 2016), and chamomile (Omer *et al.,* 2013).

In Egypt, saline water is used for irrigation in some areas. At the same time, under the arid climatic conditions prevailing in Egypt and associated with the perennial irrigation practices, imperfect drainage system, continuous increase of water table level and the relatively high salinity levels of water sources, particularly in the new reclaimed land, the salinization of Egyptian soils is rapidly becoming an acute problem (Said-Al Ahl *et al.,* 2010). However, in our opinion, this can be overcome by testing the plants' tolerance to salinity and identifying plants with the ability to adapt and have economic productivity. Therefore, the study aimed to investigate the cultivation of *Nigella sativa* in Egypt under soil salinity conditions to clarify the extent that Nigella sativa can tolerate soil salinity conditions. Since salinity is taking an increasing and threatening scale, with NaCl being the most abundant element, the objective of the study is to determine the effect of saline (NaCl) treatment on the growth, development, and production of *Nigella sativa* plants during three growth stages.

MATERIAL AND METHODS

Plant material and growing conditions

A pot experiment was conducted in a greenhouse at the Farm Station of the Faculty of Agriculture, Cairo University, Giza, Egypt, during two growing seasons. Seeds of *Nigella sativa* were obtained from Medicinal and Aromatic Research Department, Ministry of Agriculture, Egypt. The seeds were sown on 15^{th} of November, during both seasons in pots of 30 cm diameter containing 10 kg of air-dried soil. Chemical and physical analyses of the soil were conducted according to Jackson (1973). Results of the chemical analysis of the soil indicated that pH=8.40; E.C.=0.79 dSm^{-1}; total nitrogen=0.13%; available phosphorus=2.18 mg/100 g; and potassium=0.02 mg/100 g.

The soil texture consisted of 45.00% sand, 28.25% silt, 26.75% clay, and 0.85% organic matter. The experimental layout was a randomized blocks design (RBD) with 3 blocks within each season, which makes the design for the statistical analysis RBD with 6 blocks (combinations of the 3 blocks and the 2 seasons). Each block contained 10 pots of 5 plants each. The NaCl saline irrigation treatments used in the study were control (0.40 dSm^{-1}, tap water=256 ppm), 1000 ppm (1.563 dSm^{-1}), 2000 ppm (3.125 dSm^{-1}), and 3000 ppm (4.688 dSm^{-1}). The use of salt solutions started one month after sowing. Data on survival, growth characteristics, and yield components were measured during three stages of development as follows; (1) survival (%), plant height (cm), number of branches/plant, fresh and dry stem weights (g/plant), fresh and dry leaf weights (g/plant), and total fresh and dry weights (g/plant) at the vegetative stage (75 days after sowing); (2) survival (%), plant height (cm), number of branches/plant, number of umbels/plant, fresh and dry stem weights (g/plant), fresh and dry leaf weights (g/plant), fresh and dry umbels weights (g/plant), and total fresh and dry weights (g)/plant at the flowering stage (120 days after sowing); and (3) survival (%), number of capsules/plant, dry capsule weight (g/plant), dry straw weight (g/plant), seed weight (g/plant), and total dry weight (g/plant) at the fruiting stage (165 days after sowing).

Statistical analyses

Analysis of Variance (ANOVA) of a RBD was completed to determine the presence of significant differences among saline irrigation treatments at each of the three growth stages of *Nigella sativa*. Since the experiment was designed as RBD with 3 blocks within each of the two growing seasons, the 6 combinations of the 3 original blocks and the 2 seasons were used as blocks. The saline treatments during the vegetative stage were Control, 1000 ppm, 2000 ppm, and

3000 ppm; during the flowering stage were Control, 1000 ppm, and 2000 ppm; and during the fruiting stage were Control and 1000 ppm. The number of saline treatments decreased as the growth stage of the plants change because fewer and fewer plants survived at the highest level of saline treatment.

The statistical analysis was completed using the Mixed Procedure of SAS (SAS, 2014). Since the effect of Saline treatment was highly significant (P-value < 0.01) on all response variables except dry straw weight at fruiting stage, further multiple means comparison was completed using Tukey's multiple range test at 5% level of significance and letter groupings were generated. For each response variable, the validity of model assumptions (normal distribution and constant variance assumptions on the error terms) was verified by examining the residuals as described in Montgomery (2020).

RESULTS AND DISCUSSION

The ANOVA results showed that the effect of irrigation with saline water significantly affected survival and all other growth characteristics at the vegetative stage. The multiple means comparison results shown in Tables 1 and 2 reveal that irrigation with saline water has a negative effect on all growth characteristics at the vegetative stage. The mean survival, fresh stem weight, dry leaf weight, and both fresh and dry total plant weight obtained from the four treatments were all significantly different from each other. The plants that received the control treatment gave the best values compared to the rest of the treatments. Increasing saline in the irrigation water led to a decrease in the values of all growth characteristics.

For plant height and number of branches, the differences among the treatments were significant, except between 1000 and 2000 ppm saline irrigation treatments. Generally, plants treated with tap water (Control treatment) gave the best results compared to the rest of the treatments. Also, plants irrigated with 3000 ppm NaCl gave the lowest mean values of plant height and number of branches.

As shown in Table 1, there was a significant difference between the mean fresh weight of leaves from the control treatment and the rest of the treatments. While the differences among the other treatments were not significant, a decreasing trend was observed as the concentration of saline water increases. However, for dry stem weight, there was no significant difference between 2000 and 3000 ppm, but the difference between the control and 1000 ppm, and between 1000 and 2000/3000 ppm were significant (Table 2). In general, all the mean values of survival and all growth characteristics decreased with increasing NaCl concentration, and the best values were obtained from the control treatment at the vegetative growth stage.

Murillo-Amadot *et al.* (2006) tested twenty-five genotypes of *Vigna unguiculata* for salt-tolerance at the vegetative growth stage at salinity levels of 0, 85, and 170 mM NaCl. Also, a study by Murillo-Amadot *et al*. (2002) showed that seedling survival of *Vigna unguiculata* decreases linearly as salinity increases. A study by Kheloufi *et al.* (2019) on *Acacia karroo* reported that the gemination % under 400 mM NaCl decreased by up to 66%, while the seed gemination % of *Acacia saligna* at 150 mM decreased by only 18%. Cuba-Díaz *et al.* (2017) found that, NaCl decreased germination, survival seedlings % and shoot formation in *Colobanthus quitensis*. Also, NaCl inhibited germination, growth, and seed production of *Cakile maritima* (Debez *et al.*, 2004).

Table 1. Mean survival (%), plant height (cm), number of branches/plants, fresh stem weight (g/plant), and fresh leaf weight (g/plant) during the vegetative stage obtained from the 4 saline treatments.

Saline treatment (ppm)	Survival (%)	Plant height (cm)	Number of branches	Fresh stem weight (g/plant)	Fresh leaf weight (g/plant)
Control	100 a*	20.3 a	1.97 a	0.308 a	0.393 a
1000	90 b	16.2 b	1.22 b	0.191 b	0.037 b
2000	81 c	14.1 b	0.98 b	0.101 c	0.024 b
3000	48 d	11.0 c	0.69 c	0.056 d	0.015 b

**within each column, means sharing the same letter are not significantly different at the 5% level of significance*

Table 2. Mean total fresh weight (g/plant), dry stem weight (g/plant), dry leaf weight (g/plant), and total dry weight (g/plant) during the vegetative stage obtained from the 4 saline treatments.

Saline treatment (ppm)	Total fresh weight (g/plant)	Dry stem weight (g/plant)	Dry leaf weight (g/plant)	Total dry weight (g/plant)
Control	0.702 a*	0.032 a	0.039 a	0.071 a
1000	0.228 b	0.021 b	0.024 b	0.045 b
2000	0.125 c	0.012 c	0.018 c	0.029 c
3000	0.069 d	0.009 c	0.011 d	0.019 d

**within each column, means sharing the same letter are not significantly different at the 5% level of significance*

Salinity is a major abiotic stressor that restricts crop growth and development. Li *et al*. (2023) concluded that NaCl adversely affects the survival rate, growth, and development of cucumber. The study showed that cucumber plant height and growth are significantly inhibited by 150 mM NaCl, resulting in leaf wilting or even death. Li *et al*. (2023) also reported that the leaves of cucumber turn yellow under the 100 mM NaCl treatment, and most of the plants under the 150 mM NaCl treatment die. The symptom of salinity injury in plants is growth retardation due to the inhibition of cell elongation (Yasar *et al.*, 2008). Generally, the higher the salt damage index, the lower the salt tolerance. The salt damage index is divided into five levels, including normal leaf growth, drying of a small amount of the leaf edge, drying of less than 50% of the leaves, drying of more than 50% of the leaves, and entire plant death (Li *et al*., 2023). Li *et al*. (2023) also showed that salt stress significantly reduces the total chlorophyll contents. The reduced production or increased breakdown of chlorophyll molecules under salinity stress limits photosynthetic activity. Moreover, the toxicity of Na^+ or salt-induced oxidative damage triggers the disintegration of the chloroplast ultrastructure. Decreased photosynthetic pigment, stomatal conductance, impaired enzyme activity, and reduced photosynthetic activity limit the carbon fixation capacity of plants under salt stress conditions (Gul *et al.*, 2023). The photosynthetic pigments and chlorophyll contents decreased significantly due to a salt-induced increase in the activity of the chlorophyll-degrading enzyme, chlorophyllase. Lovelock and Ball (2006) explained that the reduction of carbon fixation and the biomass allocation between leaf, stem, and root could alter the balance of photosynthesis and respiration. Other possibilities related to osmotic adjustment are inability to accumulate and/or distribute sufficient nutrients or synthesize sufficient organic solutes, and the futile cycling of ions (Britto and Kronzucker 2006).

In the flowering stage, *Nigella sativa* plants were affected significantly by saline treatments. The severity of the negative effect resulted in the death of all plants irrigated with 3000 ppm NaCl. Survival, plant height, number of branches, number of umbles, and fresh and dry weights of umbles, leaves, stem as well as total weights were affected by saline irrigation. Moreover, there were significant differences among the three treatments in all growth characteristics except number of branches, where the difference between saline irrigation at 1000 ppm and 2000 ppm was not significant (Tables 3 and 4).

Table 3. Mean survival (%), plant height (cm), number of branches/plant, number of umbles/plant, fresh umbles weight (g/plant), and dry umbles weight (g/plant) during the flowering stage obtained from the 3 saline treatments.

Saline treatment (ppm)	Survival (%)	Plant height (cm)	Number of branches/ plant	Number of umbles/ plant	Fresh umbles weight (g/plant)	Dry umbles weight (g/plant)
Control	100 a*	34.5 a	5.08 a	3.78 a	0.855 a	0.177 a
1000	79 b	26.7 b	4.68 a	2.60 b	0.415 b	0.092 b
2000	27 c	18.8 c	2.78 b	1.02 c	0.147 c	0.040 c

**within each column, means sharing the same letter are not significantly different at the 5% level of significance*

Table 4. Mean fresh stem weight (g/plant), fresh leaf weight (g/plant), total fresh weight (g/plant), dry stem weight (g/plant), dry leaf weight (g/plant), and total dry weight (g/plant) during the flowering stage obtained from the 3 Saline treatments.

Saline treatment (ppm)	Fresh stem weight (g/plant)	Fresh leaf weight (g/plant)	Total fresh weight (g/plant)	Dry stem weight (g/plant)	Dry leaf weight (g/plant)	Total dry weight (g/plant)
Control	0.425 a*	0.718 a	1.66 a	0.051 a	0.112 a	0.313 a
1000	0.318 b	0.477 b	1.05 b	0.046 b	0.095 b	0.226 b
2000	0.177 c	0.238 c	0.52 c	0.035 c	0.080 c	0.155 c

**within each column, means sharing the same letter are not significantly different at the 5% level of significance*

In general, plants treated with the control treatment gave the highest values of all growth characteristics, followed by those treated with 1000 ppm NaCl, and plants treated with 2000 ppm NaCl gave the lowest values for all studied characteristics.

The negative effect of saline irrigation continued in the fruiting stage of *Nigella sativa* plants, which resulted in the death of the plants when treated with 2000 ppm NaCl. Here, at the fruiting stage only the plants that received the control and 1000 ppm NaCl survived to the end of the experiment and gave seed yield. While the differences among the treatments were significant in all studied traits (survival percentage, number of capsules, dry weights of capsules, as well as seed weight), the mean straw weights from the control and the 1000 ppm NaCl treatments were not significantly different (Table 5).

In general, the control plants gave higher mean values of all studied characteristics at fruiting stage. The results also showed that with increasing age, and increasing number of saline irrigation times, the harmful effect of salt increases because of the increased accumulation of salts in the vicinity of the roots and consequently leading to the death of plants.

Table 5. Mean survival (%), dry straw weight (g/plant), dry capsule weight (g/plant), total dry weight (g/plant), number of capsules/plant, and seed weight (g/plant) during the fruiting stage obtained from the 2 Saline treatments.

Saline treatment (ppm)	Survival (%)	Dry straw weight (g/plant)	Dry capsule weight (g/plant)	Total dry weight (g/plant)	Number of capsules/ plant	Seed weight (g/plant)
Control	100 a*	0.378 a	2.78 a	3.15 a	5.77 a	0.315 a
1000	65 b	0.233 a	2.38 b	2.61 b	3.80 b	0.207 b

**within each column, means sharing the same letter are not significantly different at the 5% level of significance*

Rebey *et al.* (2017) observed significant decreases in plant growth with increasing severity of salt treatment. Compared to the control treatment, while the application of 50 mmoL NaCl caused a slight drop (6.11%) in plant height, the application of 75 mmoL and 125 mmoL of NaCl reduced the plant height by about 32.15% and 54%, respectively. Also, they noted that salinity led to 18.5%, 35.61%, and 29.12% decline in dry matter with the application of 50, 75, and 125 mmoL of NaCl, respectively. The results indicate that salinity limits the biomass production of cumin seeds.

Results of the current study showed that, at the flowering stage, continuous irrigation with NaCl has a fatal effect, especially at concentrations of 3000 ppm or above, which led to the death of all plants. This shows that severe sensitivity of *Nigella sativa* to NaCl happens at 3000 ppm. Therefore, it is not recommended to irrigate *Nigella sativa* with 3000 ppm or above NaCl.

Salty irrigation water affects plant growth in two ways: 1) Salinity effect; plant roots take up moisture through membranes in root cells by osmosis; and water passes through a semi-permeable membrane and moves from a solution of low levels of dissolved salts to one with higher salts until the plant cells become full. If the irrigation water is moderately saline, then the growth of the plant is slowed, which leads to a reduced yield. If it is highly saline, the process of osmosis can reverse, and the plant loses moisture and suffers stress. 2) Toxicity effect; excessive concentrations of sodium and chloride ions in irrigation water can cause toxicities in plants. Sodium and chloride toxicity symptoms are leaf burn. There is another effect of sodium, which happens when sodium is high compared to calcium and magnesium leading to waterlogging because of the degradation of well-structured soils and poor plant growth (Hegazy *et al*., 2019; Sany *et al.*, 2020).

The depressive effect of salt on seed yield of several aromatic and medicinal plants including *Foeniculum vulgare* (Rahimi *et al*., 2012) and *Trachyspermum ammi* (Ashraf and Orooj, 2006) has been reported. One cause of this yield reduction under saline constraint is an inadequate photosynthesis owing to stomatal closure that limits carbon dioxide uptake (Zhu, 2001). A decrease in seed yield could be due to a reduction in flower production and/or a decrease in their fertility.

Many studies conducted on medicinal and aromatic plants concluded that the growth, productivity, and seed yield of the plants are affected negatively by saline water irrigation. Among the previous results that confirmed this are by Said-Al Ahl and Omer (2011) on different medicinal plants; Sany *et al*. (2020) on *Plectranthus amboinicus*; Hegazy *et al.* (2019) on *Satureja montana*; Said-Al Ahl *et al.* (2016) on dill; and Said-Al Ahl *et al*. (2014) on coriander. Rashed *et al.* (2017) reported that salinity treatments significantly decrease growth and yield parameters of *Nigella sativa*. Ashraf and Akhtar (2004) also reported that increasing the concentration of NaCl leads to a significant decrease in fresh and dry masses of both shoots and roots of sweet fennel. A study by Hassanzadehdelouei *et al.* (2013) showed that salinity stress has a significant inhibition of Cumin's growth stages (e.g., vegetables, flowering, and seed filling stage) where it has a significant impact on fresh weight, height and seed and yield.

CONCLUSIONS

The present work extends our knowledge about the effect of NaCl on the production of *Nigella sativa* seed, which is a source of natural products, mainly in the food industry due to its several useful compounds. From this point of view, our results revealed that NaCl decreases the seed yield of *Nigella sativa*. Since the negative impact of NaCl on the growth and development of *Nigella sativa* increases with increasing concentration of NaCl, it is recommended to irrigate *Nigella sativa* with very low concentration of NaCl. However, NaCl treatment may represent an effective method of improving the nutritional quality of *Nigella sativa* seeds through induced biochemical changes and may function as a potential source of oil and antioxidant compounds, which could support the utilization of this plant in a large field of applications including in the agroalimentary sector. Therefore, future studies should focus on working towards increasing the productivity of *Nigella sativa*, its adaptation to environmental stress, and the utilization of its biologically active compounds for therapeutic and nutritional applications as well as the development of new drugs.

REFERENCES

Arzani, A. (2008). Improving salinity tolerance in crop plants: a biotechnological view. In Vitro Cellular & Developmental Biology-Plant, 44:373-383. https://doi.org/10.1007/s11627-008-9157-7

Ashraf, M., & Akhtar, N. (2004). Influence of salt stress on growth, ion accumulation and seed oil content in sweet fennel. Biologia Plantarum, 48:461-464. https://doi.org/10.1023/B:BIOP.0000041105.89674.d1

Ashraf, M., & Orooj, A. (2006). Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). Journal of Arid Environments, 64(2):209-220. https://doi.org/10.1016/j.jaridenv.2005.04.015

Baâtour, O., Mahmoudi, H., Tarchoun, I., Nasri, N., Trabelsi, N., Kaddour, R., Zaghdoudi, M., Hamdawi, G., Ksouri, R., Lachaâl, M., & Marzouk, B. (2013). Salt effect on phenolics and antioxidant activities of Tunisian and Canadian sweet marjoram (*Origanum majorana* L.) shoots. Journal of the Science of Food and Agriculture, 93(1):134-141. https://doi.org/10.1002/jsfa.5740

Benazzouz-Smail, L., Achat, S., Brahmi, F., Bachir-Bey, M., Arab, R., Lorenzo, J. M., ... & Madani, K. (2023). Biological Properties, Phenolic Profile, and Botanical Aspect of Nigella sativa L. and Nigella damascena L. Seeds: A Comparative Study. Molecules, 28(2):571. https://doi.org/10.3390/molecules28020571

Bourgou, S., Bettaieb, I., Saidani, M., & Marzouk, B. (2010). Fatty acids, essential oil, and phenolics modifications of black cumin fruit under NaCl stress conditions. Journal of Agricultural and Food Chemistry, 58(23):12399-12406. https://doi.org/10.1021/jf103415q

Britto, D. T., & Kronzucker, H. J. (2006). Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends in Plant Science, 11(11):529-534. https://doi.org/10.1016/j.tplants.2006.09.011

Cuba-Díaz, M., Castel, K., Acuña, D., Machuca, Á, & Cid, I. (2017). Sodium chloride effect on Colobanthus quitensis seedling survival and in vitro propagation. Antarctic Science, 29(1):45-46. https://doi.org/10.1017/S0954102016000432

Debez, A., Ben Hamed, K., Grignon, C., & Abdelly, C. (2004). Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. Plant and soil, 262(1-2):179-189. https://doi.org/10.1023/B:PLSO.0000037034.47247.67

Flowers, T. J., Munns, R., & Colmer, T. D. (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of botany, 115(3):419-431. https://doi.org/10.1093/aob/mcu217

Geilfus, C. M. (2018). Chloride: from nutrient to toxicant. Plant and Cell Physiology, 59(5):877-886. https://doi.org/10.1093/pcp/pcy071

Gul, H., Ali, R., Rauf, M., Hamayun, M., Arif, M., Khan, S. A., Parveen, Z., Alrefaei, A. F., & Lee, I. J. (2023). Aspergillus welwitschiae BK Isolate Ameliorates the Physicochemical Characteristics and Mineral Profile of Maize under Salt Stress. Plants, 12(8):1703. https://doi.org/10.3390/plants12081703

Hassanzadehdelouei, M., Vazin, F., & Nadaf, J. (2013). Effect of salt stress in different satges of growth on qualitative and quantitative characteristics of cumin (*Cuminum cyminum* L.). Cercetări Agronomice în Moldova, 46:89-97.

Hegazy, M. H., Sabra, A. S., Alharbi, B. M., Said-Al Ahl, H. A., Astatkie, T., Grulova, D., & Abdelrazik, T. M. (2019). Ameliorative effects of supplemental nutrition on growth and essential oil yield of saline irrigated *Satureja montana*. Journal of Essential Oil Bearing Plants, 22(5):1218-1227. https://doi.org/10.1080/0972060X.2019.1682681

Jackson, M. L. (1973). Soil Chemical Analysis. Prentice-Hall of India, New Delhi, India.

Kabir, Y., Shirakawa, H., & Komai, M. (2019). Nutritional composition of the indigenous cultivar of black cumin seeds from Bangladesh. Progress in Nutrition, 21:428–434. https://doi.org/10.23751/pn.v21i1-S.6556

Khalil, P., Masood, S., ur Rehman, A., Iqbal, A. Z., Islam, Z., Javaid, N., Ilyas, A., Qamar, S., & Zeb, A. (2021). Proximate and sensory analysis of wheat bread supplemented with Nigella sativa oil and Nigella sativa extract. Pure and Applied Biology (PAB), 10(4):1158-1165. http://dx.doi.org/10.19045/bspab.2021.100122

Kheloufi, A., & Mansouri, L. M. (2019). Anatomical changes induced by salinity stress in root and stem of two Acacia species (*A. karroo* and *A. saligna*). Agriculture & Forestry, 65(4):137-150. https://doi.org/10.17707/AgricultForest.65.4.12

Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2016). Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). Chinese journal of natural medicines, 14(10):732-745. https://doi.org/10.1016/S1875-5364(16)30088-7

Li, L., Du, L., Cao, Q., Yang, Z., Liu, Y., Yang, H., Duan, X., & Meng, Z. (2023). Salt Tolerance Evaluation of Cucumber Germplasm under Sodium Chloride Stress. Plants, 12(16):2927. https://doi.org/10.3390/plants12162927

Lovelock, C. E., & Ball, M. C. (2006). In: Läuchli, A., Lüttge, U. (Eds.). Salinity: Environment - Plants – Molecules. Influence of salinity on photosynthesis of halophytes. Kluwer Academic Publishers, The Netherlands. p. 315–339. https://doi.org/10.1007/0-306-48155-3_15

Meloni, D. A., Oliva, M. A., Ruiz, H. A., & Martinez, C. A. (2001). Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. Journal of Plant Nutrition, 24(3):599-612. https://doi.org/10.1081/PLN-100104983

Montgomery, D. C. (2020). Design and analysis of experiments, 10th Ed. Wiley, New York, NY, USA.

Mukhtar, H., Mumtaz, M. W., Tauqeer, T., & Raza, S. A. (2021). In: Ramadan, M. F. (ed.). Black cumin (Nigella sativa) seeds: chemistry, technology, functionality, and applications, 1st edn. Composition of Nigella sativa seeds. Springer, Cham, p. 45–57. https://doi.org/10.1007/978-3-030-48798-0_5

Murillo-Amador, B., Troyo-Diéguez, E., García-Hernández, J. L., López-Aguilar, R., Avila-Serrano, N. Y., Zamora-Salgado, S., Rueda-Puente, E. O. & Kaya, C. (2006). Effect of NaCl salinity in the genotypic variation of cowpea (Vigna unguiculata) during early vegetative growth. Scientia Horticulturae, 108(4):423-431. https://doi.org/10.1016/j.scienta.2006.02.010

Murillo-Amadot, B., Troyo-Diéguez, E., López-Aguilar, R., López-Cortés, A., Tinoco-Ojanguri, C. L., Jones, H. G., & Kaya, C. (2002). Matching physiological traits and ion concentrations associated with salt stress in cowpea genotypes. Australian journal of agricultural research, 53(11):1243-1255. https://doi.org/10.1071/AR01133

Niu, Y., Zhou, L., Meng, L., Chen, S., Ma, C., Liu, Z., & Kang, W. (2020). Recent progress on chemical constituents and pharmacological effects of the genus Nigella. Evidence-based complementary and alternative medicine, 2020:6756835. https://doi.org/10.1155/2020/6756835

Omer, E. A., Said-Al-Ahl, H. A. H., El-Gendy, A. G., Shaban, K. A., & Hussein, M. S. (2013). Effect of amino acids application on production, volatile oil and chemical composition of chamomile cultivated in saline soil at sinai. Journal of Applied Sciences Research, 9(4):3006-3021.

Oyweri, J., Mohammed, A., Udu, R., Gathirwa, J., Too, E., Omondi, P., Kimani, F., Hashim, S., & Abubakar, L. (2019). In vitro and in vivo antimalarial activity of Nigella sativa L. extracts. Journal of Medicinal Plants Research, 13(19):501-508. https://doi.org/10.5897/JMPR2019.6848

Rahimi, R., Mohammolakhani, A., Roohi, V., & Armand, N. (2012). Effects of salt stress on the yield components, essential oil content and cholorophyll concentration of three fennel populations. International journal of plant production, 3:716-720.

Rashed, N., Shala, A., & Mahmoud, M. A. (2017). Alleviation of salt stress in Nigella sativa L. by gibberellic acid and rhizobacteria. Alexandria Science Exchange Journal, 38:785-799. https://dx.doi.org/10.21608/asejaiqjsae.2017.4413

Rebey, I. B., Bourgou, S., Rahali, F. Z., Msaada, K., Ksouri, R., & Marzouk, B. (2017). Relation between salt tolerance and biochemical changes in cumin (Cuminum cyminum L.) seeds. Journal of Food and Drug Analysis, 25(2):391-402. https://doi.org/10.1016/j.jfda.2016.10.001

Said-Al Ahl, H. A. H., & Hussein, M. S. (2010). Effect of water stress and potassium humate on the productivity of oregano plant using saline and fresh water irrigation. Ozean Journal of Applied Sciences, 3(1), 125-141.

Said-Al Ahl, H. A. H., & Omer, E. A. (2011). Medicinal and aromatic plants production under salt stress. A review. Herba Polonica, 57(2):72-87.

Said-Al Ahl, H. A. H., El Gendy, A. G., & Omer, E. A. (2014). Effect of ascorbic acid, salicylic acid on coriander productivity and essential oil cultivated in two different locations. Advances in Environmental Biology, 8(7):2236-2250.

Said-Al Ahl, H. A. H., El Gendy, A. G., & Omer, E. A. (2016). Humic acid and indole acetic acid affect yield and essential oil of dill grown under two different locations in Egypt. Journal of Pharmaceutical Sciences and Research, 8(7):594-606.

Said-Al Ahl, S. A., Meawad, A. A., Abou-Zeid, E. N., & Ali, M. S. (2010). Response of different basil varieties to soil salinity. International Agrophysics, 24(2):183-188.

Salehi, B., Quispe, C., Imran, M., Ul-Haq, I., Živković, J., Abu-Reidah, I. M., ... & Sharifi-Rad, J. (2021). Nigella plants–Traditional uses, bioactive phytoconstituents, preclinical and clinical studies. Frontiers in Pharmacology, 12:625386. https://doi.org/10.3389/fphar.2021.625386

Sany, H., Alharbi, B. M., Almutairi, K. A., Said-Al Ahl, H. A., Mauro, R. P., & Astatkie, T. (2020). Effects of NaCl on growth, essential oil and chemical composition of Plectranthus amboinicus. Plant Archives, 20(1):2471-2477.

SAS Institute Inc. (2014). SAS/STAT® 9.4 User’s Guide. SAS Institute Inc., Cary, NC, USA.

Shahbazi, E., Safipor, B., & Golkar, P. (2022). Responses of Nigella damascena L. and Nigella sativa L. to drought stress: Yield, fatty acid composition and antioxidant activity. Journal of Agricultural Science and Technology, 24(3):693-705.

Šutković, J., Ler, D., & Gawwad, M. R. A. (2011). Vitro production of Solasodine alkaloid in Solanum nigrum under salinity stress. Journal of Phytology, 3(1):43-49.

Taarit, M. B., Msaada, K., Hosni, K., & Marzouk, B. (2010). Changes in fatty acid and essential oil composition of sage (*Salvia officinalis* L.) leaves under NaCl stress. Food chemistry, 119(3), 951-956. https://doi.org/10.1016/j.foodchem.2009.07.055

Tarchoune, I., Baâtour, O., Harrathi, J., Hamdaoui, G., Lachaâl, M., Ouerghi, Z., & Marzouk, B. (2013). Effects of two sodium salts on fatty acid and essential oil composition of basil (Ocimum basilicum L.) leaves. Acta physiologiae plantarum, 35:2365-2372. https://doi.org/10.1007/s11738-013-1271-4

Tester, M., & Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. Annals of Botany, 91(5):503-527. https://doi.org/10.1093/aob/mcg058

Tilburt, J. C., & Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. Bulletin of the World Health Organization, 86:594-599. https://doi.org/10.2471/BLT.07.042820

WHO (2002). WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva, Switzerland.

Willcox, M. L., & Bodeker, G. (2004). Traditional herbal medicines for malaria. BMJ 329(7475):1156-1159. https://doi.org/10.1136/bmj.329.7475.1156

Yasar, F., Ellialtioglu, S., & Yildiz, K. (2008). Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. Russian Journal of Plant Physiology, 55:782-786. https://doi.org/10.1134/S1021443708060071

Zhu, J. K. (2001). Plant salt tolerance. Trends in Plant Science, 6:66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

Santa, D., Srbinovska, S., Demiri, A. (2023): Influence of starter culture and rennet in mozzarella cheese processing. Agriculture and Forestry, 69 (4): 209-220. doi:10.17707/AgricultForest.69.4.13

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INFLUENCE OF STARTER CULTURE AND RENNET IN MOZZARELLA CHEESE PROCESSING

SUMMARY

Mozzarella is well-known for its unique stretchy texture and rich flavor, making it a popular choice in various culinary applications, particularly in pizza. The aim of this study was to evaluate the influence of starter-culture and rennet on physico-chemical properties of mozzarella cheese, manufactured by an established and a new modified technology (V1: LYOFAST+MAXIREN XDS and V2:TCC-50+CHY-MAX M200). Faster fermentation was observed in V1, averaging 226 min ± 33.8, while in V2 it lasted 266 min ± 19.4. The acidity decreased in both variants, but a more pronounced downward trend in V1, and after the addition of the rennet MAXIREN XDS, a slight increase of pH was noticed. Chemical analysis demonstrated differences between the two variants, particularly in protein and fat content. V1 exhibited lower protein content compared to V2. Conversely, fat in dry matter showed significant variations, with V1 having lower values compared to V2. Additionally, V1 showed a moderately lower salt content, correlating with a lower moisture content. Higher mozzarella yield with average value of 11,90±0,20 % was achieved for V2, whereas for V1 was 11,63±1,67 %.

Keywords: mozzarella cheese, starter culture, rennet, physicochemical properties, yield

INTRODUCTION

Mozzarella is a popular unripened, soft, and white cheese known for its melting and elastic properties, making it ideal for pizzas. This is due to its high moisture content and proteins found in the curd that, when heated, form a stretchy structure. This type of cheese is representative of the "pasta filata" category of cheeses and is produced using either the direct acidification process or the more

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common use of starter culture. The direct acidification process relies on the acidification of the milk with an acid like vinegar or lemon juice. In contrast, the starter culture method relies on using a lactic acid bacteria culture to acidify the milk. The former method is more traditional and results in softer cheese, while the latter is faster and produces cheese with a more elastic texture. The use of starter cultures is crucial in producing mozzarella as it determines the cheese's sensory properties and helps create a desirable acidic environment (Varnam and Sutherland, 1994). Industries with higher capacity use concentrated cultures, lyophilized concentrated-frozen, or deep-frozen starter cultures. The amount added to a certain volume of milk is defined by the producers (Matijević *et al.*, 2015). Different starter cultures are used in mozzarella production, such as *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Enterococcus faecium* and/or *E. faecalis*, *Streptococcus thermophilus*, *L. helveticus* and *L. Delbrueckii* subsp. *Lactis* (Parente *et al*. 2017). *Streptococcus thermophilus,* which is most frequently used in the manufacturing of mozzarella, works together with *Lactobacillus delbrueckii* or *L. helveticus* to promote the growth of lactobacilli during fermentation. This starter culture rapidly generates acidity, forming a consistent curd that prevents whey separation by enzymatically breaking down lactose. Its beta-galactosidase richness enables lactose hydrolysis swiftly, particularly in an aerotolerant environment alongside *L. helveticus*. The use of *S. thermophilus* yields a cheese with reduced proteolysis, contrasting with *L. bulgaricus*, which releases multiple amino acids from casein, stimulating *S. thermophilus* growth. The interaction between these cultures supports mozzarella production (Hutkins and Ponne, 1991).

Moreover, rennet is essential to cheese production. Different types of rennet can be used depending on the cheese being made and the desired texture. *Kluyveromyces lactis* is a yeast variety known for its ability to produce beta-galactosides, enzymes that break down lactose into fermentable sugars (Johnson and Erasun, 2011). Additionally, it is capable of converting lactose into lactic acid. Alternatively, CHY MAX M 200 is a pure chymosin form obtained through submerged fermentation using the *Aspergillus niger* var. Awamori. The starter culture and rennet type determine cheese flavor, texture, and other characteristics. Additionally, other factors, such as the milk composition and the conditions under which it is processed, can also affect the final product. Moreover, the technology used in the manufacturing process of mozzarella cheese has a substantial influence on its physicochemical characteristics (Law and Tamime, 2010). Various authors have examined different categories of coagulants and their impact on the qualitative characteristics of cheeses. During the evaluation of several coagulants for the manufacturing of Halloumi cheese, it was demonstrated that Maxiren powder resulted in significantly higher levels of fat and protein losses in the whey compared to other coagulants that were evaluated (El-Zoghby and Abdel-Kader, 2000). In study by Myagkonosov *et al.* (2023) on milk-clotting enzymes for semihard cheese production, it was observed that the use of Fromase and 'Pepsin' brands resulted in significantly higher fat and dry matter losses in whey compared to Naturen, Chy-max Extra, and Chy-max Supreme clotting enzymes, even when the same amount of enzymes was used. This could potentially affect the quality and yield of the cheese.

This study aims to evaluate the impact of starter culture and rennet on the physicochemical properties of mozzarella cheese manufactured using an established technology and a new modified technology.

MATERIAL AND METHODS

In this study two variants of mozzarella cheese were examined:

a) Variant V1 - inoculated with the LYOFAST starter culture, yogurt culture *Streptococcus thermophilius* 70% and *Lactobacillus helveticus* 30%, and MAXIREN XDS rennet (which is a chymosin from the milk yeast *Kluyveromyces lactis* which is isolated from the microflora of kefir-technology applied in dairy)

b) Variant V2 - inoculated with starter culture TCC-50, which represents strains of the bacteria *Streptococcus thermophilius* and *Lactobacillus helveticus,* and followed by rennet CHY-MAX M 200

Both variations were produced on an industrial scale at a dairy facility located in Gostivar, North Macedonia. Variant 1 was already present in the dairy plant, whereas variant 2 was a newly introduced one.

The temperature and pH value were controlled throughout the technological process until the appropriate pH value was reached. Quality and chemical composition of mozzarella cheese were analysed. Analyzes of the two variants were made in four repetitions at industry level.

After 48 hours from the day of production, the following analyzes were conducted on the final products of both variants: active acidity (pH), protein content, dry matter and salt.

The following methods were used:

•for chemical-physical analyzes of mozzarella: Active acidity – pH-meter 330i WTW; Total proteins – method according to Kjeldahl (ISO 8968:1 2001); Total fat – method according to Gerber; Dry matter - by drying at a temperature of 102 °C; Moisture – gravimetric procedure; Fat in dry matter - gravimetric procedure; Salt - method according to Mohr.

Mozzarella yield was calculated according to the ratio of mass of cheese [kg] to 100 kg of milk, expressed in percentage.

Technological production procedure

The milk was pasteurized using a plate pasteurizer. The capacity of the pasteurizer was 3,000 L/h, and the milk was pasteurized at a temperature of 68 °C for a duration of 15 seconds.

Once the pasteurization process was completed, the milk was cooled to a temperature of 36 °C for variant V1 and approximately 40 °C for variant V2. The milk was then transferred to an 800 L capacity tank, where it was maintained at a temperature of 36 °C for variant V1 and around 40 °C for variant V2. Table 1 illustrates coagulation processes for V1 and V2, with differences in cooling temperatures and specific amounts of cultures and rennet added to pasteurized milk, as prescribed by the producers of rennet and starter culture.

Table 1. Process of coagulation for V1 and V2

V1:	**V2:**
Pasteurized milk – cooling to 36 °C	Pasteurized milk - cooling to 40 °C
Addition of 2.4 L of LYOFAST yogurt culture /800 L of pasteurized milk	Addition 2 L TCC 50/800 L pasteurized milk
Addition 40 ml to 45 ml of rennet to 800 L of pasteurized milk	Addition of 130 ml CHY MAX M rennet to 800 L of pasteurized milk

The milk was left to coagulate in the tank for about 40 minutes before the curd was cut into 2x2 cm pieces using a special knife shown in Figure 1. The cheese grains were then processed and dried.

Figure 1. Cutting and mixing of cheese coagulum

The cheese curd was then placed on special tables designed for whey draining (Figure 2), where it remained for approximately 3.5 hours while the whey was collected in tanks for further processing. Once the curd reached a pH of around 5.0, a stretch test was performed to determine its desired stretchability, as shown in Figure 3.

Figure 2. Whey draining

The curd was then transferred to a mozzarella machine, where it was mixed manually with water steam at a temperature of 100 °C for a period of 10 to 15 minutes. The steam used for steaming the cheese was a 3% NaCl solution. The next stage involved molding the cheese, which is illustrated in Figure 4.

Figure 3. Stretch test

Figure 4. Cheese molding

After the cheese was molded, it was cooled using cold water and then squeezed. Finally, it was packed in polyethylene (PE) bags and stored in a finished products chamber at a temperature of 2 to 4 °C with a shelf life of 7 days. The final appearance for both variants V1 and V2 is shown in Figure 5.

Figure 5. Final product

RESULTS AND DISCUSSION

The results of the fermentation process comparison between variants V1 and V2 are presented in Table 2. Variant V1, which utilized the LYOFAST starter culture, demonstrated a significantly faster fermentation process compared to V2, inoculated with TCC-50. V1 exhibited an average duration of 226 minutes ±33.8, while V2 took 266 minutes ±19.4 to complete fermentation. Notably, the shortest fermentation time observed was 200 minutes when using the yogurt culture.

Table 2. Fermentation process duration

	V1 [min]	V2 [min]
$\bar{x}$	226	266
min	200	255
max	274	295
SD	33.8	19.4

The initiation temperature for fermentation in V1 was slightly lower, around 37-38°C, compared to V2. Moreover, V1 also displayed a higher standard deviation (33.8) in fermentation duration compared to V2 (19.4), indicating greater variability in the process. Graphs 6 and 7 show the dependence of fermentation duration on temperature for the variants V1 and V2.

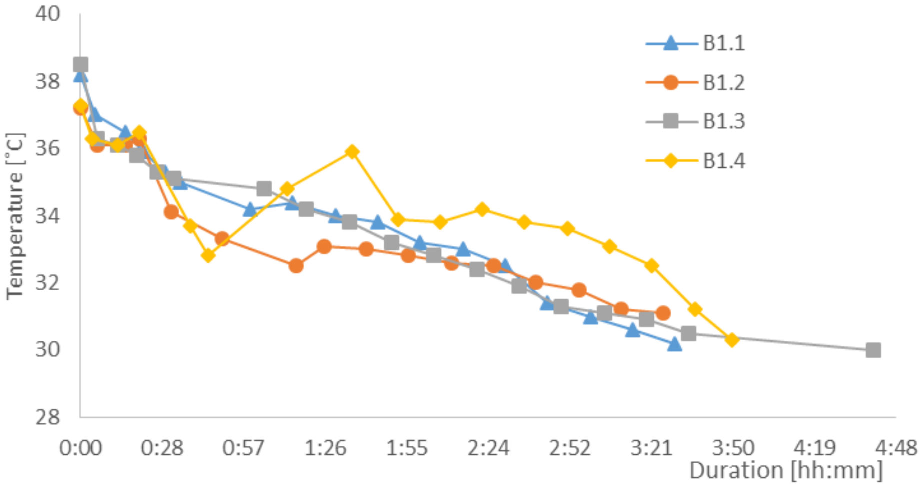

Figure 6. Temperature dependence for duration of fermentation for V1

Of particular interest was the observation that the longest fermentation duration (295 minutes) was recorded in the variant inoculated with TCC-50. This suggests that TCC-50 led to a slower fermentation process compared to the yogurt culture used in V1. Variability between repetitions was also evident, primarily attributed to the use of the LYOFAST starter culture and the storage period of the prepared working culture. The propagation process for obtaining the working culture with viable bacteria likely contributed to the variability observed in V1.

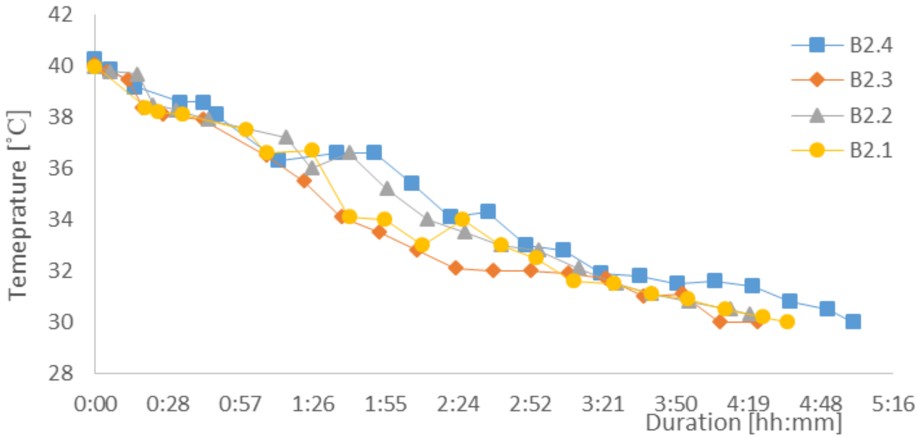

Figure 7. Temperature dependence for duration of fermentation for V2

In contrast, the TCC-50 starter culture, being a direct culture, exhibited more uniform fermentation among the four replicates. Although it had a longer fermentation period due to a higher number of active lactic acid bacteria in the latent (lag) phase, the direct inoculation approach allowed for a more standardized product.

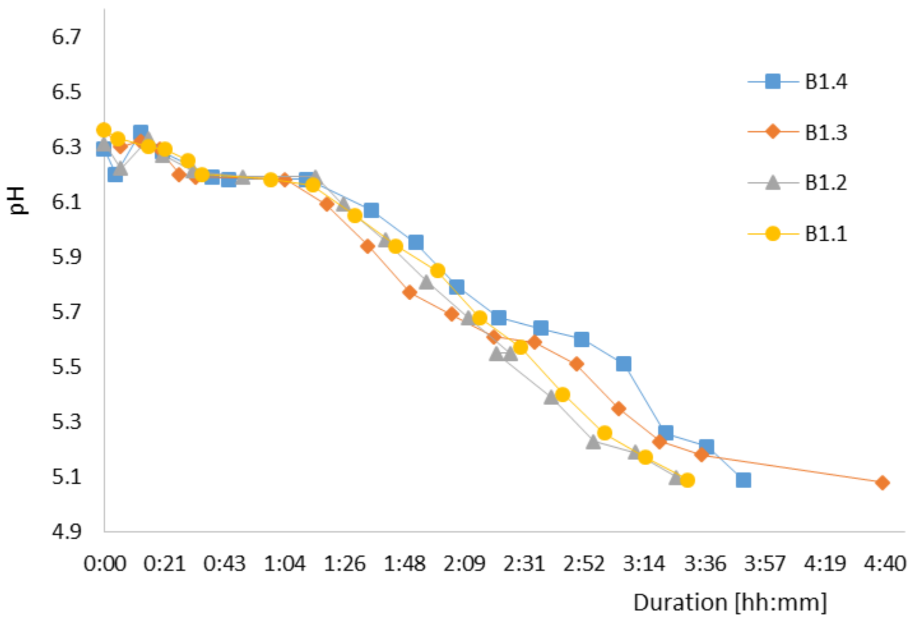

Figure 7. Dynamics of pH during the fermentation for V1

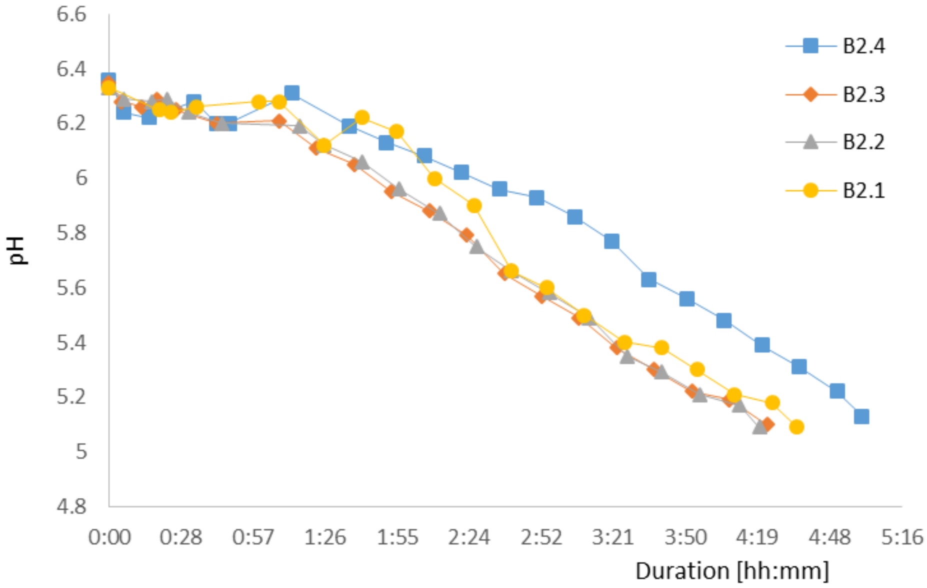

Figure 8. Dynamics of pH during the fermentation for V2

The manufacture of mozzarella is greatly influenced by pH, which affects how malleable the curd becomes in hot water or brine. Lowering curd pH (usually to 5.4–5.1) by lactose–lactic acid conversion is essential for plasticization in pasta filata cheese-making. (Guinee *et al*, 2002).

The figure 7 and a figure 8 illustrate how the duration of fermentation varies with active acidity for V1 and V2 variants, from the moment of inoculation with the starter culture to the final fermentation at pH 5.1. The acidity decreased in both variants, but a more pronounced downward trend in V1, and after the addition of the rennet MAXIREN XDS, a slight increase of pH was noticed.

The minimum initial active acidity in the variant inoculated with the LYOFAST starter culture was 6.29, with an average temperature of 37°C. In TCC-50, the initial pH ranged from 6.33 to 6.36, with an average temperature of 39.5°C. Following the inoculation of the starter culture in both variants, a reduction in active acidity was observed within a temperature range of 36°C to approximately 39°C in V1 and around 39.5°C in V2. The addition of rennet occurred ten minutes after the starter culture was added, when the milk's pH was approximately 6.3. After introducing the rennet, both the temperature and pH continued to decrease.

Upon adding the CHY MAX M 200 rennet (V2) at a temperature of about 40°C, the active acidity slightly decreased. In contrast, for V1, the active acidity increased slightly after adding the MAXIREN XDS rennet at a temperature of about 37°C, and then, after a short time, it decreased again. Throughout the whey pressing process, a continuous decrease in active acidity was observed in both variants, indicating characteristic behavior. Upon reaching a pH of 5.1, the steaming of the cheese mass commenced, followed by its formation and placement in a cold saline solution to halt the fermentation process. The results on pH are in line with the findings of Guinee et al. (2002), who investigated the effects of Ca content and pH, as well as their interaction, on the texture and heat-induced functionality of Mozzarella cheese. In comparison, Santa *et al*. (2021) reported that the average pH of the cut baskia in kashkaval, belonging to pasta filata cheeses, was 5.32±0.006. Additionally, the average active acidity of the baskia before stretching under hot brine was 5.28±0.005.

Chemical characteristics of two variants of mozzarella are presented in Table 3. The protein content showed lower values (21,20±1,38%) for V1, compared to V2 (22,74±1,19%) without significance differences, while fat in dry matter (V1: 30,85±2,36 %, V2: 37,36±1,70 %.) shows significant differences.

Furthermore, the study found that V1 had a moderately lower salt content (0.417±0.042%) compared to V2 (0.469±0.052%). This lower salt content in V1 was also correlated with a lower moisture content, which had an average value of 62,99±0.61%. In contrast, V2 exhibited higher levels of salt (0.469±0.052%) and moisture (66.29±0.78%) compared to V1.

Table 3. Chemical parameter for two variants of mozzarella cheese

Parameter	**Variant mozzarella**	$\bar{x}$	**min**	**max**	**SD**	**CV**
Protein	V1	21,20	20,05	23,20	1,38	6,5 %
	V2	22,74	21,50	24,35	1,19	5,2 %
Fat	V1	11,41	10,58	12,25	0,69	6,1 %
	V2	12,59	12,07	12,81	0,35	2,8 %
Moisture	V1	62,99	62,28	63,75	0,61	1,0 %
	V2	66,29	65,57	67,05	0,78	1,2 %
Dry matter	V1	37,01	36,25	37,72	0,61	1,6%
	V2	33,71	32,95	34,43	0,78	2,3%
Fat in dry matter	V1	30,85	28,04	33,79	2,36	7,7 %
	V2	37,36	35,14	38,87	1,70	4,5 %
Salt	V1	0,417	0,371	0,463	0,042	10 %
	V2	0,469	0,416	0,533	0,052	11 %

Significant differences in moisture, fat, and protein composition exist between the mozzarella variants (V1 and V2) examined in this study and the findings reported by Imm *et al*. (2003). The fat content of variations V1 (11.41%) and V2 (12.59%) was much lower compared to the 21.13% reported in the study conducted by Imm *et al*. (2003). Similarly, the variants exhibited a protein content that was lower than 26.07% reported in the study conducted by Imm *et al.*, (2003). In a another study conducted by Sameen et al. (2008), the fat content of mozzarella cheese was 16.50±0.50, while the protein content was found to be 14.78±0.78. In comparison to other types of cheese, such as white cheese examined in the study conducted by Jandrić and Savić (2019), the fat content on the first day of manufacture ranged from 20.43% to 22.10%.

The obtained yield for both variants is summarized through the descriptive statistics of all repetitions in Table 4. An evaluation of the yield of fresh mozzarella was conducted, with the V2 mozzarella variant showing a higher yield, averaging 11.90±0.20%. In contrast, the V1 variant had an average yield of 11.63±1.67%. The highest yield value was achieved for V2 at 12.19%, while the lowest value was recorded for V1 at 11.49%.

Table 4. Yield for variants V1 and V2

	V1		V2	
	Yield %	**L/kg**	**Yield %**	**L/kg**
$\bar{x}$	11,63 %	8,60	11,90 %	8,40
min	11,49 %	8,40	11,76 %	8,21
max	11,90 %	8,71	12,19 %	8,50
SD	0,19 %	0,14	0,20 %	0,14
Cv	1,67 %	1,65 %	1,68 %	1,66 %

To produce 1 kg of mozzarella in V1, an average of 8.6 L of milk was required, while variant 2 needed an average of 8.4 L. This suggests that implementing the technology used in producing V2 mozzarella would be more economically viable.

Earlier research by Owni and Osman (2009) found that mozzarella cheese produced from heat-treated milk had a higher yield (13.2%) compared to mozzarella produced from raw milk, which had an average yield of 11.65%. The findings of Patel *et al.* (1986) confirmed that thermal treatment of milk resulted in better retention of proteins and mineral salts, leading to higher total dry matter and yield. The increase in yield in the V2 mozzarella variant is likely attributed to denaturation and precipitation of the whey, as well as higher water retention in the soft-formed cheese, resulting in a higher content of moisture and proteins in the product.

In another study, Srbinovska *et al.* (2001) analyzed mozzarella produced from goat's milk, with fat content at 12.50±0.61%, protein at 14.04±0.21%, and total solids at 34.7±0.54%. The yield for this type of mozzarella was 18.13±0.43%, and only 5.5 L of goat's milk was needed to produce 1 kg of mozzarella, showing a significant difference when compared to the results obtained from cow's milk (8.5 L for 1 kg of mozzarella).

Various factors impact the yield of dairy products, particularly those related to the technological quality of milk and animal-related factors, such as genetics, nutrition, physiological and health status. Additionally, the actual production steps (raw material handling and curd coagulation conditions, salting, maturation, etc.) and equipment including hygiene conditions, play a crucial role (AbdEl-Gawad and Ahmed, 2011; Cipolat-Gotet *et al*., 2015; Sales *et al*., 2016). Moreover, an increase in yield can be achieved with homogenized milk, which retains more protein and fat in the curd (Kalit, 2015), while non-specific proteolytic activity can lead to a decrease in cheese yield (Matijević *et al*., 2015).

CONCLUSIONS

In summary, the objective of this study was to assess the influence of starting culture and rennet on the physicochemical characteristics of mozzarella cheese. Two variations were examined: V1, which was inoculated with LYOFAST starter culture and MAXIREN XDS rennet, and V2, which was inoculated with TCC-50 starter culture and CHY-MAX M 200 rennet. The comparison of the fermentation processes between V1 and V2 indicated that V1, which employed the LYOFAST starter culture, demonstrated a notably accelerated fermentation process in contrast to V2. The chemical examination revealed variations in the protein and fat composition, while V2 had a greater fat in dry matter, protein and salt content. Furthermore, it was seen that V2 exhibited a greater yield in comparison to V1, hence suggesting the potential economic feasibility of using the technique utilised in V2 for the manufacture of mozzarella. In conclusion, variant V2 proves to be a more appropriate option for improving mozzarella production in a dairy plant. Despite its longer fermentation period owing to a higher count of active lactic acid bacteria in the latent phase, the direct inoculation approach led to a more standardized product. Considering these factors, it is recommended for dairy plant to adopt the rennet and starter culture utilized in the second experiment for improved mozzarella manufacturing.

REFERENCES

AbdEl-Gawad, M. A. M., and N. S. Ahmed. (2011). Cheese yield as affected by some parameters review. Acta Sci. Pol. Technol. Aliment. 10:131–153

Cipolat-Gotet, C., G. Bittante, and A. Cecchinato. (2015). Phenotypic analysis of cheese yields and nutrient recoveries in the curd of buffalo milk, as measured with an individual model cheese-manufacturing process. J. DairySci. 98:633–645

El-Zoghby, A., Abdel-Kader, Y. (2000). Comparison Bettween Different Types of Coagulant for Halloumi Cheese Processing. Journal of Food and Dairy Sciences. ;25(9).

Guinee TP, Feeney EP, Auty MAE, Fox PF. (2002). Effect of pH and Calcium Concentration on Some Textural and Functional Properties of Mozzarella Cheese. Journal of Dairy Science. 85(7):1655-1669

Hutkins, R.W., Ponne, C. (1991). Lactose Uptake Driven by Galactose Efflux in Streptococcus thermophilus: Evidence for a Galactose-Lactose Antiporter. Applied and Environmental Microbiology. 7(4):941-944. doi:10.1128/aem.57.4.941-944.1991

Imm JY, Oh EJ, Han KS, Oh S, Park YW, Kim SH. (2003). Functionality and Physico-Chemical Characteristics of Bovine and Caprine Mozzarella Cheeses During Refrigerated Storage. Journal of Dairy Science. 86(9):2790-2798.

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

Johnson E., Erasun C. (2011). E., The Yeasts, Book, 5th Edition, pp.133

Kalit, S. (2015): Opče sirarstvo. kn. Sirarstvo u teoriji i praksi: 29-45

Law, B, A., Tamime A. Y. (2010). Technology of Cheesemaking. 1st ed., Wiley

Matijević,B., Rogelj I., Božanić R., Perko B., Kalit S., Barukčić I., Lisak Jakopović K., Magdić V., Stručić D., (2015). Sirarstvo u teoriji i praksi, Veleučilište u Karlovcu, Karlovac, Hrvatska, ISBN: 978-953-7343-77-4;

Myagkonosov DS, Smykov IT, Abramov DV, Delitskaya IN, Ovchinnikova EG. (2023). Influence of different milk-clotting enzymes on the process of producing semihard cheeses. Food systems. 2023;6(1):103-116.

Owni, O.A.O. El and Osman Sana, E., (2009). Evaluation of Chemical Composition and Yield of Mozzarella Cheese Using Two Different Methods of Processing. Pakistan Journal of Nutrition, 8: 684-687.

Parente, E., Cogan, M. T., Powell, B. I., (2017). Book Cheese: Chemistry, Physics & Microbiology, Chapter 8, Starter cultures: General Aspects, Fourth Edition, Volume 1, pp. 201-220

Patel, G.C., Vyas, S.H., Upadhyay, K.G., (1986). Evaluation of Mozzarella cheese made from buffalo milk using direct acidificati of technique. Int J Dairy Sci 39:394-403

Sameen, A., Anjum, F.M., Huma, N, Nawaz, H., (2008). Quality Evaluation of Mozzarella Cheese from Different Milk Sources - Open Access Library. Pakistan Journal of Nutrition. 7(6):753-756.

Santa, D., Srbinovska, S., Simonovska, T. (2021). Dynamics of acidity (pH) and temperature during the production of traditional Macedonian Galichki kashkaval. Agriculture and Forestry, 67 (3): 99-107

Sales, D. C., Rangel A. H., Urbano S. A., Borges K. C., Andrade Neto J. C., and Chagas. B. M. E. (2016). Cheese yield in Brazil: State of the art. Food Sci. Technol. (Campinas) 36:563–569

Srbinovska S., Cizbanovski T., Džabirski V., Andonov S., Palasevski B., (2001). Dynamics of salt diffusion and yield of three types of goat’s milk cheese. Mljekarstvo 51 (1) 15-26

Varnam, A.H. & J.P. Sutherland. (1994). Milk Products-1. Chapman and Hall, NewYork.

Rashev, S., Georgiev, S., Nedyalkova, S., Ivanova, S., Palagacheva, N. (2023): Pest monitoring and efficacy of plant extracts for control of cotton bollworm (Helicoverpa armigera Hübner) (Lepidoptera; Noctuidae) on sweet corn (Zea mays ssp. saccharata Sturt.). Agriculture and Forestry, 69 (4): 221-232. doi:10.17707/AgricultForest.69.4.14

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PEST MONITORING AND EFFICACY OF PLANT EXTRACTS FOR CONTROL OF COTTON BOLLWORM (*HELICOVERPA ARMIGERA* HÜBNER) (*LEPIDOPTERA*; *NOCTUIDAE*) ON SWEET CORN (*ZEA MAYS SSP. SACCHARATA* STURT.)

SUMMARY

The cultivation of sweet corn is accompanied by a number of plant protection problems. It is attacked by lot of pest, cause significant damage to the cobs, deteriorate the commercial properties and cause significant economic losses. In this regard, surveys were conducted in the period 2019-2020 in the sweet corn fields in the area of the village of Planinitsa, Burgas region, Bulgaria. Standard entomological methods were used to estimate pest densities. A mixture of plant extracts from walnut (*Juglans regia* L.), ailanthus (*Ailanthus altissima* Swing.) and tobacco (*Nicotiana tabacum* L.) was tested against cotton bollworm caterpillars (*Helicoverpa armigera* Hübner).

Sweet corn is attacked by a large number of omnivorous and specialized pests. Two critical periods are: Period I–phase 5th-7th leaf, in relation to weevils; and Period II–the paniclephenophase, when european corn borer (*Ostrinia nubilalis* Hübner) and cotton bollworm (*Helicoverpa armigera* Hübner) appear.

In 2019, 5% of cobs were reported as damaged by corn borer and 35% - by cotton bollworm, and in 2020, 10% and 65%, respectively. The data from the obtained results show that the mixture of plant extracts exhibits good efficacy and can be included as an alternative means of controlling cotton bollworm in integrated plant protection systems.

Keywords: corn, pests, *Helicoverpa armigera*, plant extracts, efficacy

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INTRODUCTION

Sweet corn is attacked by more than 141 pests that affect its vegetative and generative organs (roots, stems, leaves and cobs). They occur from sowing to harvest, resulting in reduced yield and commercial qualities (Reddy and Trivedi, 2008; Rosa *et al*., 2010).

In the initial phenophases of the cropdevelopment, serious damage to underground parts is caused by omnivorous pests: wireworms (family *Elateridae*), cutworms (family *Noctuidae*) and white worms (family *Melolonthidae*) (Li *et al*., 2004; Bessin, 2010; Foster, 2017). They spend most of their lives in the soil and can destroy young plants.

Species of genera *Agrotis, Euxoa, Noctua*, and *Spaelotis* were found among subterranean *Noctuidae* in sweet corn fields (Randall, 2019). During the day they hide in the soil and at night they feed by gnawing on the panicles and underground stems near the soil surface. The most common of them are: Turnip moth (*Agrotis segetum* Schiff.) and *Euxoa temera* Hb. (Randall, 2019).

As the crop growth progresses, the following bring a detrimental effect: maize leaf weevil (*Tanymecus dilaticollis* Gyll.), beet leaf weevil (*Tanymecus palliatus* F.), cereal leaf beetle (*Oulema melanopus* L.), corn leaf aphids (*Rhopalos iphummaidis* Fitch.) and European corn borer (*Ostrinia nubilalis* Hbn).

According to a number of authors Paulian (1972; 1978), Bărbulescu *et al*., (2001), Cristea e*t al*., (2004), Popov and Bărbulescu (2007), Georgescu *et al.*, (2014; 2018), Trotus *et al*., (2019) and Toader *et al*., (2020) the maize leaf weevil attacks more than 34 plants and is one of the key pests on sweet corn.

In the 3rd-6th leaf phenophase, the cereal leaf beetle and corn leaf aphids from family *Aphididae* pose a serious threat.

Significant damage to the generative organs is caused by: the cotton bollworm and european corn borer (Randall, 2019).

According to Li *et al*., (2004) the species: *Ostrinia furnacalis* (Guenee), *Spodoptera exigua* (Hubner), *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hubner) are pests with economic risk to the crop.

Worldwide, *H. armigera* is considered the most severe pest on sweet corn (Archer and Bynum, 1994). The cotton bollworm has been identified as a key pest on sweet corn in West Java Province, along with *Ostrinia furnacalis* Guen (*Lepidoptera; Crambidae*).

Popov and Bărbulescu (2007) identified cotton bollworm and European corn borer as key pests on sweet corn in Serbia, especially under global warming conditions. Plant damage caused by these pests greatly impairs the commercial properties (Hagerman, 1987; Hazzard and Westage, 2005), which, combined with inadequate agronomic equipment, can completely compromise the crop.

Cotton bollworm mainly prefers sweet corn rather than cotton and corn (Capinera, 2008). In some years, losses can reach up to 60-90%. The pest lays its eggs on different parts of the plant, the most being laid on the leaves -70.4%, 16.6%-on cobs, 10.4% on silk and only 2.6% on the stem (Thanee, 1987). The most serious damage is caused by the caterpillars of the pest’s second generation (Grigorov, 1976).

In a number of countries around the world (Pakistan, China, India, Australia, Thailand and Indonesia) the resistance of *H. armigera* to the insecticides used for controlwas established, therefore alternative methods and means are being researched (Daly and Murray, 1988; Ahmad *et al.*, 1988, 1997, 1998, 1999, 2001; Forester *et al.*, 1991, 1993; Armes *et al.*, 1992a, 1992b, 1994; Gunning, 1994; Gunning and Easton, 1994).

Mohammad (2019) indicated that cotton bollworm is a persistent problem for sweet corn farmers. In this regard, he tested the efficacy of various insecticides in order to reduce its population: P1 - biological insecticide (*Beauveria bassiana* 5 gl^{-1}), P2 - botanical insecticide (pandan leaves 5 $ml.l^{-1}$) and P3 - chemical insecticide (*Deltamethrin*, synthetic pyrethroid 3 $ml.l^{-1}$). Of all the applied insecticides, the highest efficacy was reported for the biological insecticide based on *Beauveria bassiana*. The best cost-effectiveness ratio was again observed with the application of the biological insecticide, followed by the botanical and lastly the chemical insecticide.

Botanical insecticides are chemicals with insecticidal properties extracted from the plants. Botanical insecticides allegedly cause little threat to the environment and almost none to plants, affect only targets insects, delay evolution of insecticide resistance, compatible with other pest control strategies, and produce healthy agricultural products free from synthetic insecticide (Subiyakto, 2011). The application of plant-based insecticides is useful in controlling caterpillars and is compatible with biological control techniques and biopesticides (Sunarto, 2009).

Several earlier studies have reported that plant extracts have successfully managed noxious insect pests Ciniviz and Mutlu (2020), Mutlu *et al.*, (2020), Teke and Mutlu (2021).

In this regard, the aim of the present study was to identify the harmful entomofauna of sweet corn and to test the efficacy of plant extracts against the cotton bollworm on sweet corn.

MATERIAL AND METHODS

The studies were conducted during the period 2019-2020 in fields sown with Syndon sweet corn hybrid, in the area of the village of Planinitsa (N 42°.89’16’’; E 27°.15’51’’) Burgas region, Bulgaria on an area of 3 ha. Observations were conducted during the entire growing season periodically, every 7-10 days. Standard entomological methods were used to report pest density.

In order to protect the beneficial species and pollinators, a mixture of plant extracts from walnut, ailanthus and tobacco was tested under field conditions.

The experiment was set up by the randomized block design in two variants, each in three replicates: I – treated and II – untreated. Observations and reports on the phenological development of the cotton bollworm in the experimental plot were carried out on 20 marked plants per variant, on the 2^{nd} and 7^{th} day after treatment, respectively.

The obtained results were processed mathematically by comparing the mean values.

RESULTS AND DISCUSSION

Harmful entomofauna in the agrocenosis of sweet corn is characterized by high species diversity. In individual years, the pests multiply massively and have a significant impact on yield formation and production quality.

In the initial phenophases of sweet corn development (sowing–germination), soil-dwelling pests pose a risk. They gnaw the germ and endosperm of the swollen seeds, of which only the shell remains. In germinated seeds, larvae damage panicles. These include: wireworms (family *Elateridae*) genus *Agriotes*, white worms (family *Melolonthidae*) genus *Melolontha*, and cutworms (family *Noctuidae*) genus *Agrotis*. In the corn crops, they were all found in mixed populations and in low density – 1-2 pcs/m^2 during the study period (Fig. 1). At the same time were found larvae of the *Pedinus femoralis* (family *Tenebrionidae*). They were counted in a higher density – 2-3 pcs/m^2

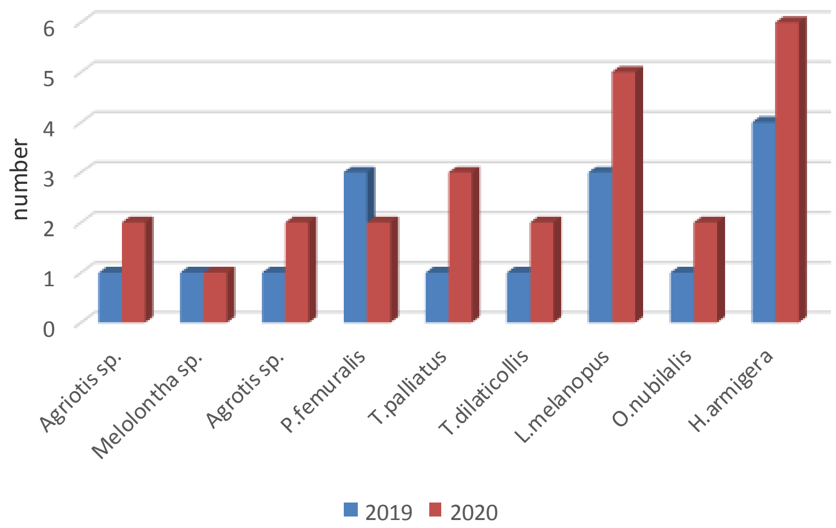

Figure 1. Species composition and density of the most significant pests of sweet corn in 2019-2020

From the emergence phase to the 5th-7th leaf of the sweet corn, serious damage was inflicted by the weevils: maize leaf weevil (*Tanymecus dilaticollis* Gyll.) and beet leaf weevil (*Tanymecus palliatus* F.). They were found in mixed populations. Their density ranged from 1 pc/m^2 in 2019 to 2-3 pcs/m^2 in 2020. At the same time, cereal leaf beetle (*Lema melanopus* L.) appeared in the sweet corn fields. The density was 3 pcs/m^2 in 2019 and 5 pcs/m^2 in 2020 (Fig. 1).

Of the sucking pests, single colonies of corn aphids, specifically *Rhopalosiphum maidis*, were found in the sweet corn fields. In the 6th-8th leaf phenophase, they occurred in low density (score 1) in both study years.

On the generative organs of sweet corn, serious damage was caused by cotton bollworm and European corn borer. The higher density among them was reported for the cotton bollworm at 4 pcs/m^2 in 2019 and 6 pcs/m^2 in 2020, respectively. The European corn borer was reported in a lower density, 1-2 pcs/m^2 in the two years of research. The young caterpillars of cotton bollworm first fed on the silk, made trails in the cobs and filled them with excrement. European corn borer caterpillars damaged the panicle, stem and cob kernels. Damage from these pests directly affected the yield and quality of produce. In 2019, the average yield was 2,300 cobs of first quality, and in 2020–1,900 cobs of first quality.

During the study period, the pest-inflicted damage to the generative organs of the plant and its reflection in the quantitative aspect were monitored.
The conducted observations showed that in 2019 the European corn borer attack was weak -5% of the damaged cobs, and 35% caused by cotton bollworm (Fig. 2). In 2019, the environmental conditions suppressed pest development and as a result the damage from them was also low. During this period, the average temperatures were lower than in 2020 and reached 24°C, whereas the maximum it was 38°C.

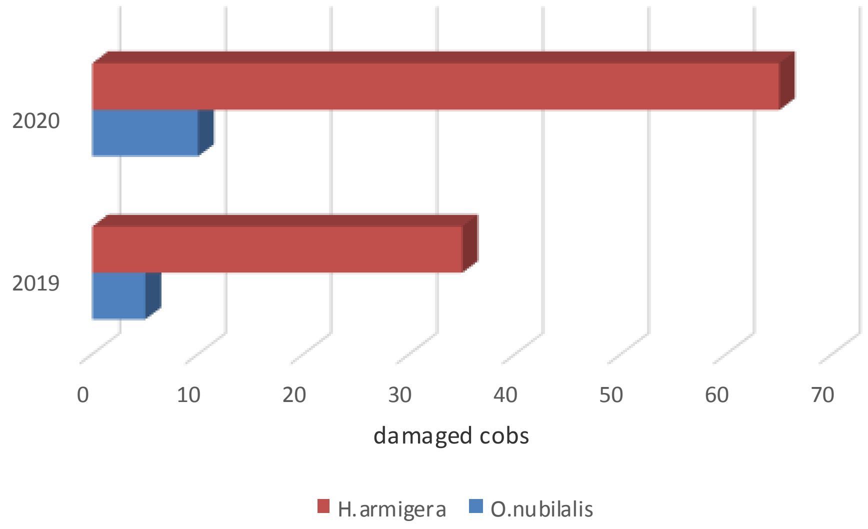

Figure 2. Cobs damaged by European corn borer and cotton bollworm caterpillars in 2019-2020.

In 2020, the European corn borer and cotton bollworm were found in higher densities, resulting in greater damage. The cob damage by the european corn borer reached 10%, and 65% inflicted by the cotton bollworm. The average temperatures for the period reached 26°C, which was due to the higher maximum temperatures approaching 40°C.

The differences in the development of European corn borer and cotton bollworm during the two study years were due to environmental factors, mainly temperature. It was higher in 2020 and, consequently, they multiplied in higher numbers and significant damages were found.

The following plant extracts from: *Juglans regia* L., *Ailantthus altissima* Swing. and *Nicotiana tabacum* L. were tested for the control of cotton bollworm.

Before treatment in the individual variants, there was no proven statistical difference, which can be seen in Table 1 and Figure 3. The calculated significance level p=0.185196 was significantly greater than the threshold p=0.05.

Table 1. Comparison of mean values from the different variants before treatment

T-test for Independent Samples (cotton bollworm before treatment)	
	treated vs. untreated
Mean1	2.833333
Mean2	3.100000
t-value	-1.33271
df	118
p	0.185196
Valid N1	60
Valid N2	60
Std.Dev.1	1.076193
Std.Dev.2	1.115378
F-ratio	1.074146
p	0.784460

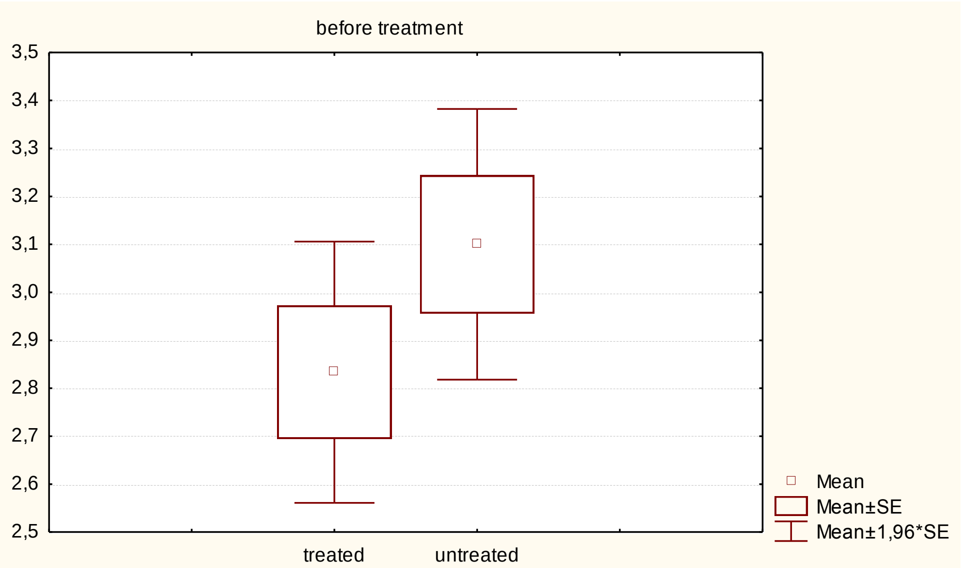

Figure 3. Comparison of mean values in the different variants before treatment

On the second and seventh days, the efficacy of the used mixture of plant extracts was clearly visible (Tables 2 and 3). The number of caterpillars in the treated plots was statistically proven to be lower than in the untreated ones. On both the second and the seventh day, the level of significance (p=0.000000) was significantly lower than the marginally acceptable (p=0.05). The above stated is clearly visualized in Figures 4 and 5.

Table 2. Comparison of mean values from the different variants on the 2nd day

T-test for Independent Samples (cotton bollworm after 48 h)	
	treated vs. untreated
Mean1	1.450000
Mean2	3.100000
t-value	-9.43565
df	118
p	0.000000
Valid N1	60
Valid N2	60
Std.Dev.1	0.768556
Std.Dev.2	1.115378
F-ratio	2.106169
p	0.004850

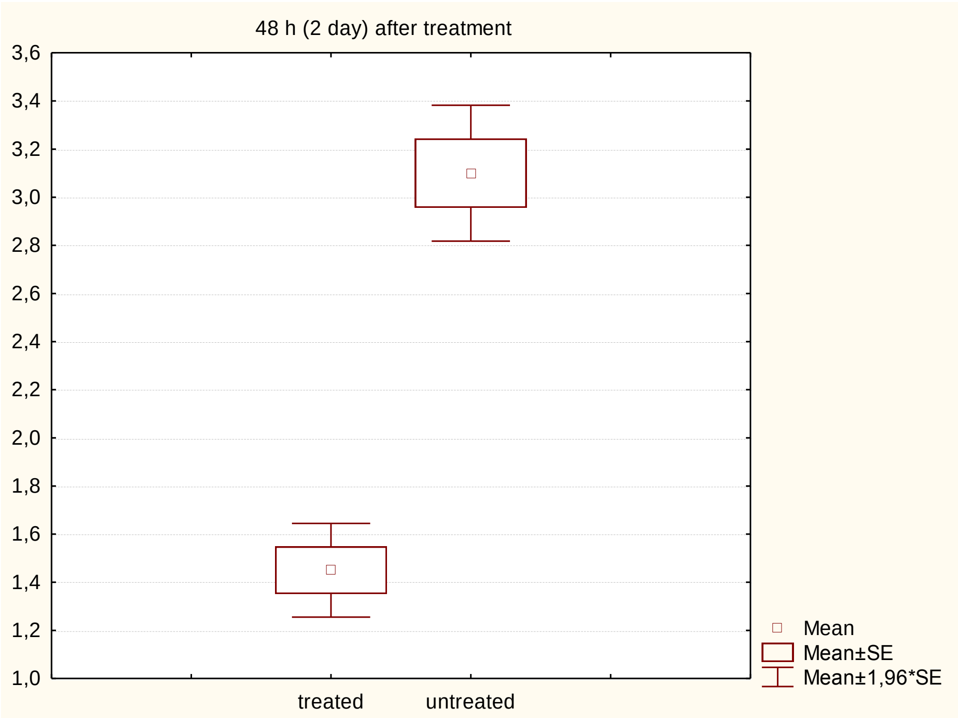

Figure 4. Comparison of mean values of results obtained on the 2nd day after treatment

Table 3. Comparison of mean values from the different variants on the 7th day

T-test for Independent Samples (cotton bollworm after 168 h) Note: Variables were treated as independent samples	
	treated vs. untreated
Mean1	0.333333
Mean2	3.100000
t-value	-17.6752
df	118
p	0.000000
Valid N1	60
Valid N2	60
Std.Dev.1	0.475383
Std.Dev.2	1.115378
F-ratio	5.505000
p	0.004850

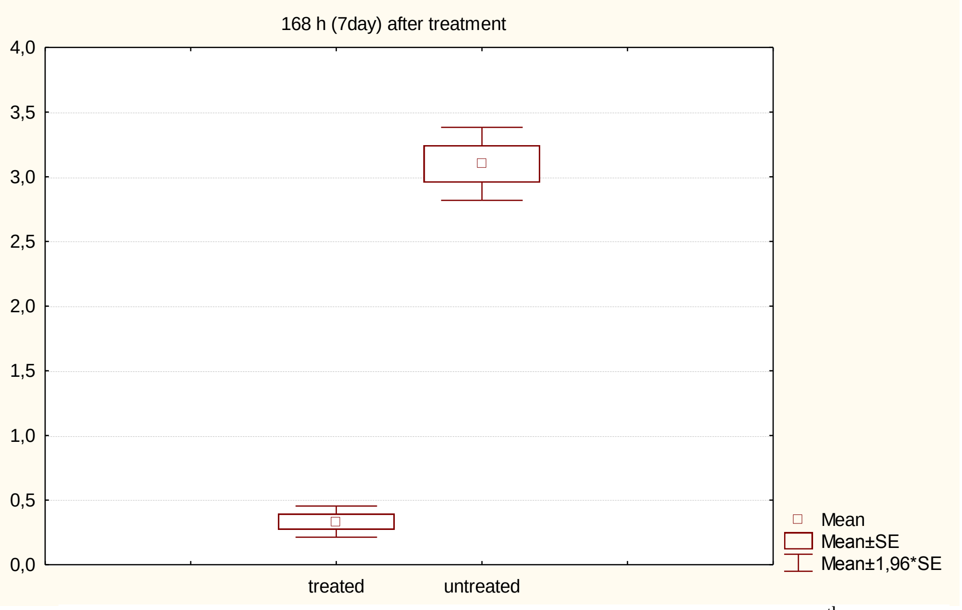

Figure 5. Comparison of mean values of results obtained on the 7th day after treatment

The used mixture of plant extracts can be applied in integrated plant protection systems against cotton bollworm, while protecting the beneficial entomofauna, pollinators and not contaminating the produce.

The results of the study confirm the results of Archer and Bynum (1994), Capinera (2008), Mohammad Y. (2019), according to which the cotton bollworm is a main pest of sweet corn and causes significant damage. Its caterpillars gnaw the grains in the cobs, and when they feed on the cob silk, they prevent pollination of the plants.

The obtained experimental data regarding the plant extracts are in sync with those cited in the literature. Kamaraj *et al*., (2008), Ali *et al.*, (2021) tested the efficacy of plant extracts of *Citrus sinensis*, *Ocimum canum, Ocimum sanctum*, *Rhinacanthus nasutus*, *Azadirachta indica*, *Curcuma* sp., *Allium sativum,* and *Polygonum hydropiper* against *H. armigera* and found that, that they all exhibit good efficacy against the enemy. The authors recommend their use in practice as an ecological approach to cotton bollworm control.

CONCLUSIONS

As a result of the conducted research, the following conclusions can be drawn:

•Sweet corn is attacked by lot of number pest. The critical periods for control of sweet corn enemies have been established (I period - from the germination phase to the 5-7th leaf, the gray corn borer (*Tanymecus dilaticollis* Gyll.) and the gray beet borer are a serious danger (*Tanymecus palliatus* F.) and II period - phenophase sweeping, serious damage is caused by the corn stem borer (*Ostrinia nubilalis* Hübner) and the cotton bollworm (*Helicoverpa armigera* Hübner.).

•In 2019, the European corn borer and the cotton bollworm were found to be in low density. 5% damaged cobs by the corn stem borer and 35% of the cotton bollworm was reported. In 2020, the environmental conditions favored the multiplication and development of the enemies, as a result of which a higher percentage of damaged cobs was recorded 10% of the corn borer, respectively 65% of the cotton bollworm.

•The mixture of plant extracts from: walnut (*Juglans regia* L.), wild walnut (*Ailantthus altissima* Swing.) and tobacco (*Nicotiana tabacum* L.) show good efficacy against cotton bollworm caterpillars. The obtained results are a prerequisite for the application of the plant extracts in the integrated control systems with the key enemy of sweet corn, in which the environment, the beneficial entomofauna and the pollinators of the cultivated plants are protected.

REFERENCES

Ahmad, M., Arif, A.I., Ahmad, Z. & Attique, M.R. (1998). *Helicoverpa armigera* resistance to insecticides in Pakistan. In: Beltwide Cotton Conference (National Cotton Council of America). p.1138-1140.

Ahmad, M., Arif, M. I. & Ahmad, Z. (1999). Patterns of resistance to organophosphate insecticides in field populations of *Helicoverpa armigera* in Pakistan. Pestic. Sci. 55: 626-632.

Ahmad, M., Arif, M. I. & Ahmad, Z. (2001). Resistance to carbamate insecticides in *Helicoverpa armigera* (*Lepidoptera: Noctuidae*) in Pakistan. Crop Protection. 20: 427-432.

Ahmad, M., Arif, M.I. & Attique, M.R. (1997). Pyrethroid resistance of *Helicoverpa armigera* (*Lepidoptera, Noctuidae*) in Pakistan. Bull. Entomol. Res. 87: 343-347.

Ahmad, M. & McCaffery, A.R. (1988). Resistance to insecticides in a Thailand strain of *Heliothis armigera* (Hübner) (*Lepidoptera: Noctuidae*). J. Econ. Entomol. 81: 45-48.

Ali, S., Li, Y., Haq, I.U., Abbas, W., Shabbir, M.Z. & Khan, M.M. (2021). The impact of different plant extracts on population suppression of *Helicoverpa armigera* (Hub.) and tomato (*Lycopersicon esculentum* Mill) yield under field conditions. PLoS ONE 16(12): e0260470. (available at https://doi.org/10.1371/journal.pone.0260470)

Archer, T.L. & Bynum, E.D. (1994). Corn earworm (*Lepidoptera: Noctuidae*) biology on food corn on the high plains. Environ Entomol. 23: 343-348.

Armes, N.J., Banerjee, S.K., Desouza, K.R., Jadhav, D.R., King, A.B.S., Kranthi, K.R., Regupathy, A. & Surulivelu, T.N.V.R. (1994). Insecticide resistance in *Helicoverpa armigera* in India: recent developments. In Brighton Crop Protection Conference: Pests and Diseases. British Crop Protection Council. Brighton, UK. p.437-442.

Armes, N.J., Jadhav, D.R., Bond, G.S. & King, A.B.S. (1992a). Insecticide resistance in *Helicoverpa armigera* in South India. Pestic. Sci. 34: 355-364.

Armes, N.J., Jadhav, D.R. & King, A.B.S. (1992b). Pyrethroid resistance in the pod borer, *Helicoverpa armigera* in southern India. In: Brighton Crop Protection Conference: Pests and Diseases. British Crop Protection Council, Brighton, UK. P 239-244

Bărbulescu, A., Voinescu, I., Sadagorschi, D., Penescu A., Popov, C. & Vasilescu, S. (2001). Cruieser 350 FS-A new product for maize and sunflower seed treatment against *Tanymecus dilaticolis* Gyll. Romanian Agricultural Research, 15, 77-87.

Bessin, R. (2010). Sweet corn pests. Extension Entomologist University of Kentucky College of Agriculture.

Capinera, J.L. (2008). Maize (Corn) pests and their management. In J. L. *Capinera* (ed.), Encyclopedia of entomology, 2nd ed., Springer Science+Business Media B.V. Dordrecht, the Netherlands. pp. 2267–2269

Ciniviz, G. & Mutlu, Ç. (2020). Effectiveness of some native diatomaceous earth against maize weevil, *Sitophilus zeamais* (*Coleoptera: Curculionidae*), under controlled conditions. Agriculture & Forestry/Poljoprivreda i Sumarstvo, 66(4), 151-162. (available at DOI:10.17707/AgricultForest.66.4.12).

Cristea, M., Căbulea, I. & Sarca, T., (2004). Maize-Monographic study (Porumbul-Studiu monografic). Romanian Academy Publishing House, Chapter 1: 589-626.

Daly, J.C. & Murray, D.A.H. (1988). Evolution of resistance to pyrethroids in *Heliothis armigera* (Hübner) (*Lepidoptera: Noctuidae*) in Australia. J. Econ. Entomol. 81: 984-988.

Forrester, N.W. (1991). Pyrethroid and endosulfan resistance in *Heliothis armigera* in Australia 1990-1991. Resistant Pest Management. 3: 31-34.

Forrester, N.W., Cahill, M., Bird, L.J. & Layland, J.K. (1993). Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (*Lepidoptera: Noctuidae*) in Australia UK. CAB International. pp. 132.

Foster, R. (2017). Managing Insects in Commercially Grown Sweet Corn. Extension Entomology. Purdue University. E-98-W (available at. www.extension.purdue.edu 1-888-EXT-INFO).

Georgescu, E., Cană, L., Popov, C., Gărgăriță, R., Râșnoveanu, L. & Voinea, L. 2014. Maize leaf weevil (*Tanymecus dilaticollis* Gyll) in the context of neonicotinoid seed treatment restriction. Annals of N.A.R.D.I. Fundulea, 82, 251-277.

Georgescu, E., Crețu, A., Zob, C. & Cană, L. (2018). Preliminary results concerning maize leaf weevil (*Tanymecus dilaticollis* Gyll) control, in commercial farm conditions, from south-east of the Romania. Scientific Papers. Series Agronomy, 61(1), 221-226.

Grigorov, S. (1976). Special enthomology. State Publishing House for Agricultural Literature. pp. 60. (In Bulgarian)

Gunning, R.V. (1994). Pyrethroid resistance increasing in unsprayed populations of *Helicoverpa armigera* (Huebner) (*Lepidoptera: Noctuidae*) in New South Wales 1987-90. J. Aust. Entomol. Soc. 33: 381-383.

Gunning, R. V. & Easton,C. S., 1994. Endosulfan resistance in *Helicoverpa armigera* (Hubner) (*Lepidoptera: Noctuidae*) in Australia. J. Aust. Entomol. Soc. 33: 9-12.

Hagerman, P. (1987). Revision of "European Corn Borer in Sweet Corn and Other Horticultural Crops," December 1997, 97-019.

Hazzard, R. & Westgate, P. (2005). Organic Insect Management in Sweet Corn. Scouting, thresholds and management methods for key caterpillar pests. SARE Outreach.

Kamaraj, C., Rahuman, A.A. & Bagavan, A. (2008). Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). Parasitol Res 103, 1361–1368 (2008). (available at https://doi.org/10.1007/s00436-008-1142-x)

Li, D.S., Zhang, B.X., Huang, S.H. & Feng L. (2004). The occurrence and damages of insect pests on sweet corn in Guangdong Province. Acta Phytophylacica Sinica, Vol.31, No.1, p. 6-12.

Mohammad, Y. (2019). Efficacy of biological insecticides against *'Helicoverpa armigera'* in sweet corn crop (*'Zea mays* saccharata') [online]. Australian Journal of Crop Science, Vol. 13, No. 2, Feb 2019: 321-327. (available at : https://search.informit.com.au/documentSummary;dn=352578786964247;res=IELHSS>ISSN: 1835-2693. [cited 15 Mar 20]).

Mutlu, Ç.,Çiftçi, V., Yeken, M. Z. & Mamay, M. (2020). The influence of different intensities of chalky spot damage on seed germination, grain yield and economic returns of red lentil. Phytoparasitica, 48(2), 191–202.

Paulian, F. (1972). Contribution at knowledge of the development, ecology and control of the *Tanymecus dilaticollisspecie* (Contribuţii la cunoaşterea dezvoltării, ecologiei şi combaterii speciei *Tanymecus dilaticollis*). Doctoral thesis, I.A.N.B. Bucharest, chapter IV: 49-57, chapter IV: 193-200, chapter IV: 209-225.

Paulian, F. (1978). Maize pests and heir control (Dăunătorii porumbului şi combaterea lor). Academy of Agricultural Sciences, Agricultural Library, Bucharest: 1-32.

Popov, C. & Bărbulescu, A. (2007). 50 years of scientific activity in field protection domain against diseases and pests. Annals of N.A.R.D.I. Fundulea, 75, 371-404.

Randall, R. (2019). Insect Pest of Sweet Corn. Home&Garden Information Centre, USA.

Reddy, Y.V.R. & Trivedi, S., (2008). Maize Production Technology. Academic Press, USA, pp.192

Rosa, A.P.S.A., Martins, J.F.S., Porto, M.P., Schafer, J.T., Chevarria, V.V., Chaves, C.C. & Pinto, C.C. (2010). Ocorrência da lagarta-do-cartucho em função da época de semeadura do milho nas safras 2007/2008 e 2008/2009. Pelotas: Embrapa Clima Temperado.

Subiyakto, S. (2011). Teknologi Pengendalian Hama Berbasis Ekologi dalam Mendukung Pengembangan Kapas. SJJPdPP; 30(3):81–86.

Sunarto, D.A. (2009). Peran insektisida botani ekstrak biji mimba untuk konservasi musuh alami dalam pengelolaan serangga hama kapas. Jurnal Entomologi Indonesia, 6(1):42–42.

Teke, M.A. & Mutlu, Ç. (2021). Insecticidal and behavioral effects of some plant essential oils against *Sitophilus granarius* L. and *Tribolium castaneum* (Herbst). J Plant Dis Prot 128, 109–119. (available at https://doi.org/10.1007/s41348-020-00377-z).

Thanee, N. (1987). Oviposition preferences and larval food quality of *Helicoverpa armigera* (H.). Massey University; Palmerson, New Zealand (Ph.D. thesis)

Toader, M., Georgescu, E., Ionescu, A.M. & Șonea, C. (2020). Test of some insecticides for Tanymecus dilaticollis Gyll. control, in organic agriculture conditions. Romanian Biotechnological Letters, 25(6), 2070-2078.

Trotuș, E., Mincea, C., Dudoiu, R., Pintilie, P.L. & Georgescu, E.I. (2019). The preliminary results regarding the impact of the neonicotinoids insecticides, applied at rape, sunflower and maize seedtreatment, on the harmful entomofauna and honey bees. Annals of N.A.R.D.I. Fundulea, 87, 251-260.

Osmani, I., Mula, V., Hashani, Z., Bajraktari, D., Zeneli, L. (2023): Heavy metals levels in raw cow's milk and health risk implication from thermal power plants emission in Obilic, Kosova. Agriculture and Forestry, 69 (4): 233-244. doi:10.17707/AgricultForest.69.4.15

DOI: 10.17707/AgricultForest.69.4.15

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HEAVY METALS LEVELS IN RAW COW'S MILK AND HEALTH RISK IMPLICATION FROM THERMAL POWER PLANTS EMISSION IN OBILIC, KOSOVA

SUMMARY

Contamination of food reflects the most serious consequences of environmental contamination, whereas cow's milk is considered the most representative food source contaminated with heavy metals. The main objective of this study is to evaluate the level of toxic heavy metals (Cd, Pb) and nutrition elements (K, Na, Ca, Mg, Cu, Fe, Cr, Zn) in cow milk collected from different sites in the Industrial area of thermal power plant in Obilic, Kosova. To determine the level of presence of chemical elements, total of 153 cow milk samples are collected from 51 cows. From each location sites during the morning milking in the different seasons [spring (phase I), summer (phase II), and autumn (phase III)]. Elements were determined using SAA atomic spectrometry. The results of this study indicate that heavy metals content extend the recommended values by International Dairy Federation and Codex Alimentarius Commission in all three phases of monitoring, and also there is a strong correlation between heavy metals and nutritional elements in cow's milk.

Keyword: heavy metals, nutrition elements, milk, correlation

INTRODUCTION

Due to its medicinal and dietary properties, the consumption of cow's milk is very popular in the world. This is also because the beneficial health effects are beyond its value. However, very little is currently known about the distribution, behavior and effects of trace elements in cow's milk. Industrial progress and the increase in agricultural production cause the use of large amounts of chemicals even in the production of animal feed, therefore agricultural production has

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become a permanent source of external chemicals for living organisms (Zeneli *et al.*, 2013). An important "direct indicator" of the uncontaminated condition of milk is determination of residual metal concentrations in milk. More ever, it can be also an "indirect indicator" of the degree of pollution of the environment from origin of milk (Licata *et al.*, 2004, González-Montaña *et al.*, 2012). The problem with metals is their ability to bioaccumulate, and in recent decades, contamination of milk with metals has been considered a dangerous problem. Heavy metals come into contact with animals through air, water and food, where after ingestion of unhealthy foods is considered the main source of metal residues in secreted milk.

Therefore, milk as a secretion of the mammary gland together with the many substances it carries, constitute a risk factor for the health of the consumer. Milk and most of the dairy products from cows are likely to be exposed to heavy metal contamination during lactation period (Ogut *et al*., 2016). In particular, metal residues in milk remain a concern due to consumption by infants and children.

Lead and cadmium cause the greatest concern in terms of adverse effects on human health (Zeneli *et al.*, 2008). The risk is also increased by the fact that they are easily transferred through food chains and are not known for essential biological functions. Therefore, the concentration of these two metals should be observe in cow's milk to guarantee the health of people (Fathi *et al*., 2020). On the other hand, milk is known as an excellent source of calcium and can supply zinc, iron and copper in smaller amounts (Pennigton *et al*., 1995).

Although the excretion of metals through milk is very low, the accumulation of their ecosystem (water-soil-plant-animal) makes them very toxic and harmful to us living organisms (Tunegova *et al.*, 2016).

According to conducted research's, milk and milk products contain around 38 micro and different trace elements. Some of them are essential and very important, because they are cofactors in many enzymes and play an important role in many physiological functions of humans and animals. The amount of metals in uncontaminated milk is undoubtedly small, but their content can be significantly altered and cause serious health problems (Amer *et al*., 2021). Depending on several factors, the content of these minerals in raw cow's milk can vary, e.g. lactation period, presence of any disease, seasonal variations, climatic conditions, annual feed composition and environmental pollution (Licata *et al*., 2004, Yahaya *et al*., 2010). Therefore, data and research related to the concentration of heavy metals in cow's milk is important in assessing the risk to human health. Cadmium and lead accumulation in ruminants induce toxic effects in cattle, therefore also in humans who consume contaminated food (including milk) with toxic metals (González-Weller et al., 2006, Cai *et al*. 2009, Vromman *et al*., 2008).

According to previous studies, it was determined that the content of selected pollutants in the study area differ among elements and samples, and they can cause harmful effects in plants, animals and humans (Bajraktari *et al*. 2019, Zeneli *et al.*, 2011). Therefore, the aim of this study was to determine the residue levels of toxic heavy metals (Cd, Pb) and nutrition elements (K, Na, Ca, Mg, Cu, Fe, Cr, Zn) in Industrial area of Obilic, Kosova. It should be noted that toxic metal contamination in milk has been proven also in different countries.

MATERIAL AND METHODS

A total of 153 fresh cow milk samples (each sample 500 mL) were collected from 51 cows in villages selected in area of Kosovo Thermal Power Plants in Obilic Municipality (Figure 2). Samples was taken during the morning milking directly in sterile bottle to prevent any contamination. The milk samples from cow were carried in three phases (spring, summer and autumn). Transportation of samples into laboratory was carried immediately (at 4 °C).

Before the procedure, the working glassware are wet with detergent, rinsed with tap water. The same are moistened with 15% nitric acid, rinsed with distilled water and stored in the oven at 110 ^{o} C as needed. From each sample, 0.5 g of each milk samples was taken and digested with 5 mL of concentrated nitric acid. Content of flask was heated at 80 ^{o} C, until the clear digest was gained. The excess acid was evaporated. Cooling take place at room temperature, and the final solution was dilute to 25 mL with 0.2 mol/L nitric acid and filtered. Quantitative determination of selected elements and were conducted by using atomic absorption spectrometer (Jen *et al*., 1994). Also, blank solutions were also analyzed with same method. Data were statistically evaluated and concentrations were expressed as mean ± standard deviation, minimum and maximum values.

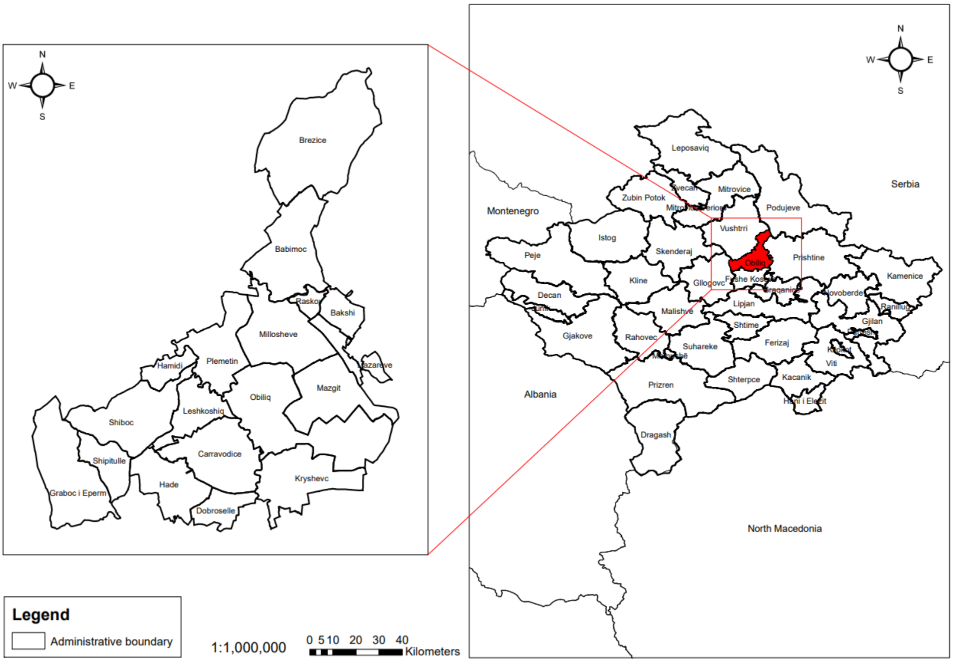

Figure 1. The map of Kosova and the Municipality of Obilic - The location of the Kosovo Thermopower Plants

RESULTS

The concentration of nutritional elements in milk samples during three phases showing the minimum, maximum, and average values for the analyzed elements in each phase are given in in Table 1, 2 and 3. Regarding the mean values of nutrition elements, they differ from sample to sample, and from one phase to another one. Iron mean concentration in phase one was 0.587 mg/L with minimum concentration 0.314 mg/L and maximum concentration 0.836 mg/L, which are lower compared with Phase II detected iron in milk samples. In phase two the mean iron concentration was 0.716 mg/L, with minimum concentration 0.218 mg/L and maximum concentration was 2.5 higher compared with phase I (2.042 mg/L). In phase three mean iron concentration was 0.467 mg/L, with minimum concentration lower than in phase one and two (0.047mg/L) and maximum concentration 1.041 mg/L. Copper mean concentration as nutritional elements varies from phase to phase, with lowest mean concentration [0.297 mg/L (phase I); 0.361 mg/L (phase II); and 0.170 mg/L (phase III)]. Maximum concentration was detected in phase II (5.085 mg/L) and lowest in phase III (0.59 mg/L).

Table 1. Mean, minimum, and maximum contents of selected elements in milk samples – Phase I

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Mean ± SD	0.587±0.15	0.297±0.08	0.307±0.12	3.064±0.41	0.291±0.11	33.909±5.11	9.758±1.28	20.217±1.82	0.318±0.11	1.293±0.52
Median	0.622	0.331	0.352	3.05	0.292	34.486	9.818	19.808	0.34	1.429
Minimum	0.314	0.156	0.019	2.337	0.159	22.701	6.705	17.25	0.104	0.144
Maximum	0.836	0.429	0.648	4.155	0.914	42.138	12.174	26.564	0.482	2.097
Confidence Level (95.0%)	0.048	0.027	0.076	0.133	0.037	1.646	0.412	0.585	0.037	0.167

Mean chromium concentration various from phase to phase. Regarding the maximum concentration, the highest value was detected in phase one samples (0.648 mg/L) while in phase two and three we have almost the same result (0.474 mg/L vs 0.481 mg/L). Mean magnesium concentration differ from phase one with higher value of 3.064 mg/L, to lower value in phase two 1.56 mg/L and phase three 1.221 mg/L. Zinc minimum concentration was detected in phase three (0.012 mg/L), and maximum zinc concentration was detected in phase one (0.914 mg/L) with mean concentration varying from 0.291 mg/L in phase one, to 0.251 mg/L in phase two and lowest mean concentration in phase three (0.048 mg/L). Because numerous biological and environmental samples contain low concentration of zinc, it is not difficult to contaminate them (Guyo *et al.*, 2009). Studies also demonstrated that Zn concentrations in mammary tissue were positively correlated with the milk performance and age of dairy cows (Olsson *et al.*, 2001). Potassium mean concentration various from 33.909 mg/L (phase one) and 31.242 mg/L (phase two), with lowest mean concentration in phase three 19.085 mg/L. Minimum concentration was detected in phase three (0.139 mg/L) and maximum concentration was detected in phase one 42.138 mg/L.

Table 2. Mean, minimum, and maximum contents of selected elements in milk samples – Phase II

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Mean ±SD	0.726±0.34	0.361±0.09	0.278±0.117	1.565±0.15	0.251±0.13	31.24±6.02	7.15±3.27	14.83±8.86	0.236±0.06	1.625±0.67
Median	0.78	0.166	0.33	2.135	0.262	32.64	8.093	19.358	0.247	1.534
Minimum	0.218	0.026	0.056	0.072	0.041	5.078	0.067	1.682	0.109	0.511
Maximum	2.042	5.085	0.474	3.227	0.722	38.024	10.852	25.419	0.346	2.844
Confidence Level (95.0%)	0.125	0.327	0.061	0.554	0.049	2.209	1.2	3.251	0.028	0.286

Sodium mean concentration values differ from phase one 9.758 mg/L to 7.15 mg/L in phase two and 6.45 mg/L in phase three, with minimum concentration in phase two (0.067 mg/L) and maximum concentration in phase one (12. 174 mg/L), which do not differ much from phase two and three (10.852 mg/L, respectively 11.309 mg/L). Calcium minimum and maximum concentration was detected in phase three 0.303 mg/L respectively 35.092 mg/L, with mean concentration various from 20.217 mg/L in phase one, followed by 14.838 mg/L in phase two and 17.035 mg/L in phase three. Calcium concentration in milk, similarly to the Mg content from high-producing cows often falls below the lower reference limits (Zamberlin *et al.* 2012, Litwinczuk *et al.,* 2018). Base on many authors, milk from cows which are suffering from mastitis, has lower calcium proportion than milk of the highest cytological quality (Kowalczuk *et al*., 2007, Górska *et al.*, 2012, Bilandžić *et al.*, 2011).

Table 3. Mean, minimum, and maximum contents of selected elements in milk samples – Phase III

	Fe mg/L	Cu mg/L	Cr mg/L	Mg mg/L	Zn mg/L	K mg/L	Na mg/L	Ca mg/L	Cd mg/L	Pb mg/L
Mean ± SD	0.467±0.27	0.170±0.02	0.073±0.02	1.221±0.24	0.048±0.08	19.09±9.08	6.45±1.67	17.03±7.87	0.313±0.09	2.277±1.07
Median	0.477	0.206	0.103	1.897	0.051	22.203	5.832	18.604	0.337	2.282
Minimum	0.074	0.059	0.022	0.048	0.012	0.139	4.231	0.030	0.100	0.367
Maximum	1.041	0.379	0.481	4.479	0.206	27.739	11.309	35.092	0.426	3.803
Confidence Level (95.0%)	0.105	0.077	0.089	0.549	0.031	3.455	0.635	2.996	0.034	0.41

Regarding the concentration of toxic heavy metals (Cd and Pb) in milk samples during three phases are given in Figure 2 and mean, minimum and maximum concentrations in Table 2, 4 and 6.

Mean concentration of cadmium and lead in phase one was 0.318 mg/L, respectively 1.293 mg/L, followed with mean concentration for cadmium in phase two (0.236 mg/L) and lead (1.625 mg/L). Higher mean concentration was detected for lead in phase three (2.277 mg/L), while for cadmium we have almost the same mean concentration in phase three as in two previous phases (0.313 mg/L). Minimum cadmium detected concentration was almost the same in all three phases (0.104 mg/L; 0.109 mg/L; 0.100 mg/L) while maximum concentration of cadmium various from 0.482mg/L in phase one, to 0.346 mg/L in phase two and 0.426 mg/L in phase three. Lead minimum concentration was detected in phase one with 0.144 mg/L, followed with phase two 0.511 mg/L a phase three 0.367 mg/L. Maximum concentration of lead was detected in phase three with 3.803 mg/L, while in phase one maximum detected value was 2.097 mg/L followed with 2.844 mg/L in phase two. These results are higher than values reported by Simsek *et al*. (2000).

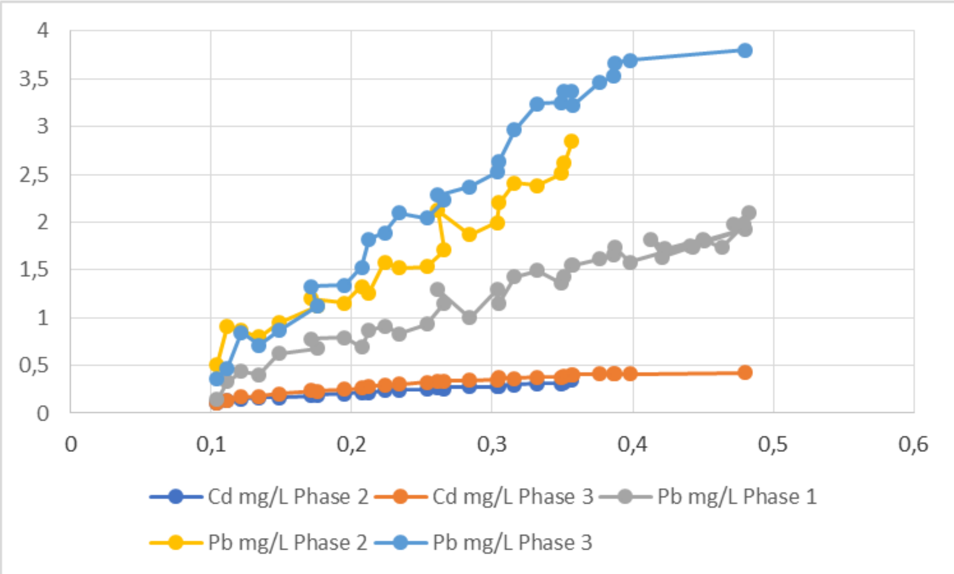

Figure 2. Concentration of Cd and Pb (mg/mL) in three phases

Table 4. Correlation between heavy metals and nutrition elements in Phase I

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Fe (mg/L)	1									
Cu (mg/L)	**0.875**	1								
Cr (mg/L)	**0.735**	**0.752**	1							
Mg (mg/L)				1						
Zn (mg/L)	0.192	0.155	0.183		1					
K (mg/L)	0.121	0.077			0.169	1				
Na (mg/L)				0.299	0.073		1			
Ca (mg/L)			0.023	**0.54**			0.117	1		
Cd (mg/L)	**0.876**	**0.929**	**0.806**		0.171	0.051			1	
Pb (mg/L)	**0.829**	**0.923**	**0.761**		0.132	0.022			**0.981**	1

The values indicate significance at $p < 0.001$

The Pearson correlation matrix, provided in Table 4, 5 and 6, shows significant correlation between heavy toxic metals and nutritional elements selected for analysis in each phase of sampling. In phase one iron is strongly positive correlated with cadmium and lead (r=0876, respectively r=0.829), and copper with cadmium and lead (r=0.929, respectively r=0.923). Also, in phase one cadmium and lead revealed high positive correlation with chromium and calcium.

Table 5. Correlation between heavy metals and nutrition elements in Phase II

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Fe (mg/L)	1									
Cu (mg/L)	0.15	1								
Cr (mg/L)	0.291	0.289	1							
Mg (mg/L)		0.097	0.021	1						
Zn (mg/L)	0.199	0.116	0.343	0.11	1					
K (mg/L)		0.182		0.26		1				
Na (mg/L)	0.073	0.139	0.071	0.107	0.393		1			
Ca (mg/L)				0.207		0.268		1		
Cd (mg/L)	0.131	0.334	**0.976**	0.215	0.132				1	
Pb (mg/L)	0.253	0.421	**0.949**	0.237	0.204				**0.973**	1

The values indicate significance at $p < 0.001$

In phase two regarding heavy toxic metals and nutritional elements, the Pearson correlation matrix revealed significant positive correlations between cadmium (r=0.976), and lead (r= 949) with chromium.

Table 6. Correlation between heavy metals and nutrition elements in Phase III

	Fe mg/L	Cu mg/L	Cr mg/L	Mg mg/L	Zn mg/L	K mg/L	Na mg/L	Ca mg/L	Cd mg/L	Pb mg/L
Fe mg/L	1									
Cu mg/L	**0.689**	1								
Cr mg/L	**0.861**	**0.634**	1							
Mg mg/L		0.127		1						
Zn mg/L	0.354	0.446	0.413	0.258	1					
K mg/L				0.382	0.095	1				
Na mg/L	0.001	0.062	-0.07	0.325	0.031	0.017	1			
Ca mg/L				0.156		0.04	0.261	1		
Cd mg/L	**0.703**	**0.642**	**0.894**		**0.513**				1	
Pb mg/L	**0.788**	**0.647**	**0.949**		0.481				**0.978**	1

The values indicate significance at $p < 0.001$

The data in Table 6 indicate significant positive correlations (Pearson coefficients) between heavy toxic metals and nutritional elements. Cadmium and lead shows strong positive correlation with iron (r=0.703; r=0.788) and chromium (r=0.894, r=0.949). Cadmium revealed moderate positive correlation with cooper (r=0.642) and zinc (r=0.513).

DISCUSSION

The statistical analysis shows difference in the concentration of nutritional elements and cadmium and lead depending on the sampling time as shown in Figure 2. Iron concentration in phase one was 0.314 mg/L-0.836 mg/L, in phase two concentration various from 0.218 mg/L to 2.042 mg/L. and in phase three iron concentration various from 0.047 mg/L to 1.041 mg/L, which are higher maximal concentration compared with result presented by Pilarczyk *et al.* (2013).

The mean value of iron in all three phases was higher than results represent by Pilarczyk *et al*. (2013) which various from 0.1984 mg/L to 0.2576 mg/L. Copper mean concentration as nutritional elements varies from phase to phase, with maximum concentration detected in phase II (5.085 mg/L) which is higher concentration of copper in milk samples than results present by Mahlat *et al*. (2012) (2.836 µg/g). Chromium concentration various from phase to phase, but it was lower than mean results presented by Cocho *et al*. (1992).

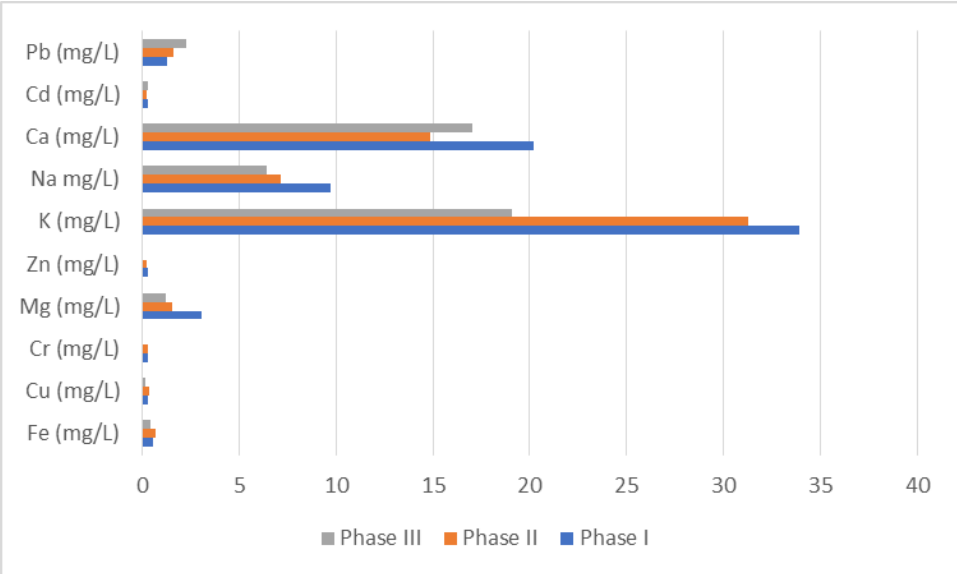

Figure 3. Mean concentration of selected elements distributed in three phases

Mean magnesium concentration differ from phase one with higher value of 3.064 mg/L, to lower value in phase to 1.56 mg/L and phase three 1.221 mg/L, which are higher than results presented by Gaucheron (2005) which are 1.043–1.283 mg/L. Zinc maximum concentration and mean values were lower than those presented by Malhat *et al.* (2012). Potassium maximum mean concentration was detected in phase one 33.909mg/L (phase one), which is lower significantly than those presented by Gaucheron (2005) 1212–1681 mg/L. Also, sodium content with higher mean concentration in phase one (9.758 mg/L) was much lower than previous results presented by Gaucheron (2005) which various 391–644 mg/L. Calcium maximum concentration was detected in phase three 35.092 mg/L which is significantly lower than results presented by Nogalska *et al.* (2020).

Mean nutritional content in the milk samples in all location sites in phase one followed the profile K > Ca > Na > Mg > Fe > Cr > Cu > Zn. In phase two nutritional elements follow almost the same profile: K > Ca > Na > Mg > Fe > Cu > Cr > Zn. In phase three nutritional elements follow this rank K > Ca > Na > Mg > Fe > Cu > Cr > Zn. Based on the above results the nutritional elements follow almost the same profile. This is also the same profile as presented by Naeem *et al.* (2022), in which zinc is switched with iron.

Regarding the toxic heavy metals, there was no significant seasonal variations in cadmium content, compared to reports by Kozhanova *et al.* (2021) where was detected significant seasonal variations in cadmium content in animal milk. In all three seasons the maximum level and mean value of cadmium and lead exceeds the values recommended in these reports and indicate a concentration in toxic values (IDF Standard, 1979, Codex Alimentarius Commission, 2007). Mean heavy metal content in the milk samples in all location sites in phase one followed the profile lead > cadmium. Correlations between milk components are presented in each phase in Table 4, 5 and 6.

A strong correlation between heavy metals and nutritional elements in cow's milk, in all three phases are observed respectively for cadmium and lead with iron, copper and chromium, moderate correlation with magnesium and zinc. The results show a very high correlation in iron and cadmium compared to cases in plants (Bajraktari *et al.,* 2020). There is noted in phase one besides a correlation for heavy metals and nutrients elements, a high negative correlation between iron with copper (r=-0.875), high positive correlation between iron and chromium (r=0.735) and copper and chromium (r=0.752), moderate positive correlation between magnesium and calcium (r=0.540), and very high positive correlation between cadmium and lead (r=0.981). In phase two, besides high positive correlation of chromium with cadmium and lead, there is detected a very high positive correlation between cadmium and lead (r=0.973), and very high negative correlation between lead and chromium (r=-0.949). In addition to heavy metal and nutritional elements correlations, in phase three there is noted a very high positive correlation between cadmium and lead (r=0.978), iron with chromium and copper (r=0.861, r=0.689), copper with chromium (r=0.634). Correlations between nutritional milk elements and toxic metals were rarely analyzed (Rodríguez *et al.*, 1999). As in the milk of Simmental and Holstein-Friesian cows, where significant high positive correlations were found between the concentrations: Pb–Cd (r=0.86 vs. r=0.87), during three phase of study also strong positive correlation between cadmium and lead was detected (Pilarczyk *et al.,* 2013).

CONCLUSIONS

The aim of this study was to analyze the content of nutritional elements in various seasonal sampling time and heavy toxic metals in cow's milk in highly polluted industrial area and to compare the results of previous studies. Results suggested that cadmium and lead concentration was higher than the recommended standards and compared with results of milk from other parts of the world. The present research showed that cow's milk in three phases of sampling had advantageous nutritional composition and higher toxic heavy metal concentration. The established correlations showed high significantly positive correlation between toxic heavy metals (cadmium and lead) in all three with chromium, in phase one and three with iron, copper. The comparative analysis in this study suggests taking the necessary measures for the production of safe food products of animal origin.

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REFERENCES

Zeneli, L., Daci- Ajvazi, M., Daci, N.M., Hoxha, D., & Agron Shala (2013). Environmental Pollution and Relationship Between Total Antioxidant Capacity and Heavy Metals (Pb, Cd, Zn, Mn, and Fe) in Solanum tuberosum L. and Allium cepa L., Human and Ecological Risk Assessment: An International Journal, 19(6), 1618-1627.

Licata, P., Trombetta, D., Cristani, M., Giofrè, F., Martino, D., Calò, M., et al. (2004). Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environment International, 30(1), 1–6.

González-Montaña, J. R., Senís, E., Gutiérrez, A., & Prieto, F. (2012). Cadmium and lead in bovine milk in the mining area of the Caudal River (Spain). Environmental Monitoring and Assessment, 184(7), 4029–4034.

Ogut, H. Secilmis Canbay, H. (2016). Effect of Environmental Factors on Heavy Metal Content of Raw Milk. Akademik Gıda, 14(2), 105 -110

Zeneli, L., Daci, N.M., Daci-Ajvazi, M.N., Paçarizi, H. (2008). Effects of pollution on lead and cadmium concentration and correlation with biochemical parameters in blood of human population nearby Kosovo thermo power plants. American Journal of Biochemistry and Biotechnology, 4(3), 273–276.

Fathi, M., Khalili, N., Rezaei, N. (2020). Effects of lead and cadmium on the immune system and cancer progression. Journal of Environmental Health Science and Engineering, 18(1), 335-343.

Pennigton, A., Schoen, A., Salmon, D., Young, B., Johnson, D., Marts, W. (1995). Composition of core foods of the US food supply, 1982–1991. J Food Compos Anal 8:171–217

Tunegova, M., Toman, R., Tančin, V. (2016). Heavy Metals – Environmental Contaminants And Their Occurrence In Different Types Of Milk, Slovak J. Anim. Sci., 49(3): 122–131

Amer, A.A.E., El-Makarem, H.S.A., El-Maghraby, M.A., Abou-Alella, S.A. (2021). Lead, cadmium, and aluminum in raw bovine milk: Residue level, estimated intake, and fate during artisanal dairy manufacture. Journal of Advanced Veterinary and Animaly Research, 8(3):454-464.

Yahaya, M.I., Ezo, G.C., Musa, Y.F., Muhamad, S.Y. (2010). Analysis of heavy metals concentration in roadside soils in Yauri, Nigeria. Afr J Pure Appl Chem. 4(3):022–30.

González-Weller, D., Karlsson, L., Caballero, A., Hernández, F., Gutiérrez, A., González-Iglesias, T., et al. (2006). Lead and cadmium in meat and meat products consumed by the population in Tenerife Island, Spain. Food Additives and Contaminants, 23(8), 757–763.

Cai, Q., Long, M. L., Zhu, M., Zhou, Q. Z., Zhang, L., & Liu, J. (2009). Food chain transfer of cadmium and lead to cattle in a lead–zinc smelter in Guizhou, China. Environmental Pollution, 157(11), 3078–3082.

Vromman, V., Saegerman, C., Pussemier, L., Huyghebaert, A., De Temmerman, L., Pizzolon, J. C., et al. (2008). Cadmium in the food chain near non-ferrous metal production sites. Food Additives and Contaminants: Part A, 25(3), 293–301.

Bajraktari, D.; Bauer, B.; Zeneli, L. (2019) Environmental pollution and heavy metals accumulation in Salix alba L. (Fam. Salicaceae), along the river stream of Sitnica. Agric Conspec Sci 84:95–101

Zeneli, L., Daci, N.M., Paçarizi, H., Daci-Ajvazi, M. (2011). Impact of Environmental Pollution on Human Health of the Population Which Lives Nearby Kosovo Thermopower Plants. Indoor and Built Environment. 20(4), 479-482.

Simsek, O., Gültekin, R., Öksüz, O., & Kurultay, S. (2000). The effect of environmental pollution on the heavy metal content of raw milk. Nahrung, 44(5), 360–363.

Bilandžić, N., Dokić, M., Sedak, M., Božica, S., Varenina, I., Knežević, Z., et al. (2011). Trace element levels in raw milk from northern and southern regions of Croatia. Food Chemistry, 127(1), 63–66.

Jen, S. L., Lee, S. J., & Lin, S. Y. (1994). Determination of cadmium and lead in raw milk by graphite furnace atomic absorption spectrophotometer. Journal of Dairy Science, 77(4), 945–949.

Guyo, H., Saegerman, C., Lebreton, P., Sandersen, C., Rollin, F. (2009). Epidemiology of trace elements deficiencies in Belgian beef and dairy cattle herds. Journal of Trace Elements in Medicine and Biology 23: 116–123.

Olsson, I.M., Jonsson, S., Oskarsson, A. (2001). Cadmium and zinc in kidney, liver, muscle and mammary tissue from dairy cows in conventional and organic farming. J. Environ. Monit. 3, 531–538.

Zamberlin, Š., Antunac, N., Havranek, J., Samaržija, D. (2012). Mineral elements in milk and dairy products. Mljekarstvo, 62, 111–125

Litwinczuk, Z., Koperska, N., Chabuz, W., Kedzierska-Matysek, M. (2018). Basic chemical composition and mineral content of the milk of cows of various breeds raised on organic farms and on traditional farms using intensive and traditional feeding systems. Medycyna Weterynaryjna, 74, 309–313.

Kowalczuk, E., Klebaniuk, R. (2007). Nutritional approach to mastitis prevention. Higiena, 2, 12–14.

Górska, A., Mróz, B. E. (2012). Effect of the inflammation of the mammary gland on the content of macro- and microelements in milk. Medycyna Weterynaryjna, 68, 697–699.

Pilarczyk, R., Wójcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., Tomza-Marciniak, A. (2013). Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environmental Monitoring and Assessment, 185: 8383–8392.

Malhat, F., Hagag, M., Saber, A., Elsalam Fayz, A. (2012). Contamination of Cows Milk by Heavy Metal in Egypt. Bulletin of Environmental Contamination and Toxicology, 88:611–613.

Cocho, J.A., Cervilla, J.R., Rey-Goldar, M.L., Fdez-Lorenzo, J. R., Fraga, J. M. (1992). Chromium content in human milk, cow's milk, and infant formulas. Biological Trace Element Research. 32: 105-107.

Gaucheron, F. (2005). The minerals of milk. Reproduction Nutrition Development, 45(4), 473–483.

Nogalska, A., Momot, M., Nogalski, Z. (2020). The Mineral Composition of Milk from High-Yielding Dairy Cows Depending on the Month of Lactation and Udder Health. Appl. Sci., 10, 4803.

Naeem, S., Nazir, A., Imran, M., Hussain, S M. (2022). Development and storage stability of conjugated linoleic acid fortified yogurt. Food Science and Technology (Campinas), 42(1) DOI: 10.1590/fst.110121

Kozhanova, N., Sarsembayeva, N., Lozowicka, B., Kozhanov, Zh. (2021). Seasonal content of heavy metals in the “soil–feed–milk–manure” system in horse husbandry in Kazakhstan. Veterinary World, 14(11): 2947-2956.

IDF Standard. (1979). Metal contamination in milk and milk products. International Dairy Federation Bulletin. Document no. A. Doe 37

Codex Alimentarius Commission. (2007). Joint FAO/WHO food standards programme. Codex committee on methods of analysis and sampling, twenty-eighth session, Budapest, Hungary, 5–9 March 2007

Bajraktari, D., Bauer, B., Zeneli, L. et al (2020). Soil chemical evaluation and power plant ash impact on chemical properties of Salix alba L. (Fam. Salicaceae): The impact of bioaccumulation. Toxicology Research and Application 4(4).

Rodríguez Rodríguez, E. M., Sanz Alaejos, M., & Díaz Romero, C. (1999). Chemometric studies of several minerals in milks. Journal of Agricultural and Food Chemistry, 47 (4), 1520–1524.

Jokanović, O., Radonjić, D., Marković, B., Mirecki, S., Veljić, M. (2023): Sensory characteristics of Kučki cheese. Agriculture and Forestry, 69 (4): 245-252. doi:10.17707/AgricultForest.69.4.16

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SENSORY CHARACTERISTICS OF KUČKI CHEESE

SUMMARY

The physical, chemical, microbiological and sensory analysis of the food products is used for the quality evaluation. But in parallel, sensory properties of food are equally important. Sensory analysis is usually used as a tool to improve the sensory quality of products and to achieve their standard quality. The paper presents the results of sensory analysis of the three types of cheese: cheese made of cow's milk; ewe's milk; and mixed milk (cow's+ewe's). The following characteristics: taste, odour, consistency and color, outer appearance, and cut were evaluated. The sensory analysis was performed using the scoring system and the sensory evaluation of cheese quality. The cheeses were graded from 1 to 5 points. The grades were multiplied by appropriate factors to obtain scores for each cheese parameter. The maximum number of points is 20. There are five categories of quality and quality ranges from "unacceptable" to "excellent". The results of sensory analysis showed that all three types of cheese had good quality. The best-rated type of cheeses is ewe's milk cheese, got 18.45 points, out of a maximum of 20, which classified it as cheese with excellent quality category, especially when the tastes and cross-sectional appearance are in consideration. The cheese made from mixed milk obtained 17.63 points, that classified, also, it into the group of excellent quality category cheese while the cow's cheese had 17.08 points, classified as good quality category cheeses.

Keywords: sensory characteristics, cheese, ewe's and cow's milk

INTRODUCTION

Due to their chemical composition and physicochemical characteristics, most foods are very perishable and are easily altered by physical, chemical and biological agents (Martinez *et al*., 2021). Sensory analysis comprises a variety of powerful and sensitive tools to measure human responses to foods and other products. Sensory quality has been recognized as a crucial aspect of the sale and marketing of cheese (Gulzat *et al*., 2020), it is now generally accepted that the

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flavour of most cheese results from the combination of a large number of several compounds present in the correct ratios and concentrations, which is known as component balance theory (Fatma *et al*., 2013). Cheese is a milk product characterized by a wide range of sensory properties that directly influence its consumption. During the ripening of cheese, numerous chemical, biochemical, physical and microbiological changes occur, that directly contribute to its sensory properties. Thus, sensory analysis of cheese as a final product is used for its quality evaluation. Cheeses from cows fed on mountain pastures had a greater variety of tastes (Buchin *et al*., 1998). According to El-Nimr *et al.* (2010) texture and color are important criteria used to evaluate cheese quality; these two parameters are often the primary consideration of consumers when making purchasing decisions.

White-brine cheeses are one of the most commonly produced cheeses whose production is widespread in the world. Also, they are one of the oldest types of cheeses, and Dozet. (2004) states that the production of these cheeses was recorded in the region of Egypt more than 3200 years BC. Low-fat white cheese has a harder, coarser dough, whereas cheeses with higher milk fat content have a softer, creamier consistency (Živković, 1971).

Cattle husbandry in Montenegro is the most important branch of animal husbandry and agriculture in general. Annual production of cow's milk is about 180 thousand tons. Breed composition of Montenegrin cattle population is quite unfavorable because various crossbreeds dominate, about 50% of the total population. In the last two decades share of high productive breeds Holstein and Simmental has been increased, at the same time Brown Swiss and Grey cattle sharply decreased, while autochthonous Busa breed is in risk of extinction (Marković *et al*., 2021). Natural grasslands have a special importance in Montenegro, because their share in the total agricultural area is above 90% and they are often the only source of fodder for ruminants (Dubljević *et al.*, 2020). The sheep production is mainly based on rearing autochthonous breeds. Jezeropivska pramenka is one of the most important autochthonous Montenegrin sheep breeds, and made about 20% of its total sheep population (Đokić *et al.*, 2020).

The original technology and specificity of the raw milk are the most important characteristics for traditional cheeses. Golijski cheese must have a mildly salty, sour flavor with a hint of young walnut flavor. This taste is stronger at first; in cross-section, it may just have a few circular holes or none at all. It is a cheese with little pressing, so mechanical cavities are obvious (Ostojić *et al*., 2010). According to Jovanović *et al.* (2004), a slice of Sjenički cheese has an undamaged regular shape, a distinctive white color, cheese curd of medium hardness that has a modest amount of cavities on the cut. The odour is typical, with nice milky-sour notes and, in certain examples, a stronger saltiness. After 20 days of ripening, Domiati type cheeses grow even harder, saltier, and have a more pronounced taste and fragrance (Tratnik *et al*., 2000). According to results obtained by Carvalho *et al.* (2020) more mature ewe’s milk cheeses are given higher scores for their characteristic and pungent attributes of flavor. Ewe’s milk cheeses made from raw milk, such as those used in this study, are firmer and have a more characteristic odor, taste, and aftertaste than those manufactured with pasteurized milk (Mendia *et al.*, 1999). The original technology and specificity of the raw milk are the most important characteristics for traditional cheeses (Santa *et al.*, 2021), sheep’s milk has high values of chemical components, and it is the best raw material for the production of cheese, because it gives twice higher yield than cow’s milk (Jandrić and Savić, 2019).

Kučki cheese has a distinctive aroma, taste, and consistency, specific traditional technology, the production area is defined and, as such, meets the requirements for the protection of origin. Production of Kučki cheese is an inseparable part of the heritage of the Kuči region, based on a family tradition, which is passed down from generation to generation and represents an unavoidable part of history and the material treasure of the mentioned area (Jokanović *et al.*, 2021).

The aim of this scientific paper is to collect adequate data for sensory evaluation of Kučki cheese quality made from cow's, ewe's, and mixed milk. Kučki cheese belongs to a valuable group of cheeses in brine, is a part of cultural heritage of this area of Montenegro that should be protected, standarised and better valorised. In recent years, the area of Kuči has increasing number of tourists who come from all over the world not only to enjoy the natural beauty, but to consume the traditional products of this region. In addition to the economic benefit for the population of this area and the preservation of Kučki cheese technology, the protection of origin of this product would also be a significant form of stimulus for traditional agricultural production, which contributes to the preservation of the environment, the protection of biological diversity, and the protection and better valorization of rural areas.

MATERIAL AND METHODS

A total of 15 samples of Kučki cheese from 3 groups made from cow's, ewe's, and mixed milk), 5 samples of each were taken for sensory evaluation. The cheeses were produced in the Kuči area, north of the Montenegrin capital of Podgorica with surrounding mountains that have natural conditions for sheep and cattle production. The most of milk is used for the production of traditional Kučki cheese, that is full-fat, white brine cheese. The ripening in the brine gives cheese specific sensory properties and the possibility to be stored for a longer period, different from other white-brined cheeses produced in Montenegro.

The sensory evaluation of cheese quality was monitored using the scoring system according to Ritz (1991). Each group was evaluated separately, and the groups were classified according to their evaluations and compared. The maximum score that a cheese can get with such appraisal is 20 weighted points.

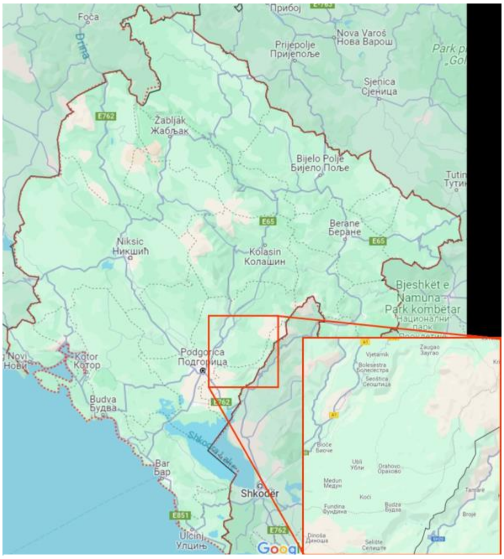

Figure 1. The area of Kučki cheese production

From the table 1, it could be seen that the cheese is graded from 0 to 5 points with the application of significance factors for each individual parameter. The obtained grades multiplied by the significance factor give the corresponding number of weighted points and the following six sensory parameters were appraised as shown in table 1.

Table 1. The scoring system for cheese sensory evaluation

Parameter	Points (a)	Factor (b)	Maximum points (a x b)
Appearance	0-5	0.4	2.0
Color	0-5	0.2	1.0
Consistency	0-5	0.4	2.0
Cut	0-5	0.6	3.0
Odour	0-5	0.4	2.0
Taste	0-5	2.0	10.0
		TOTAL POINTS:	20.00

There are five categories of quality category: excellent (17.6-20); good (15.2-17.5); mediocre (13.2-15.1); still acceptable (11.2-13.1) and not acceptable (<11.2) points. The commission for sensory evaluation of cheese was a trained panel - staff of the Dairy Laboratory. The data were processed using the computer program STATISTICA 12. An analysis of variance was performed using One-way ANOVA for the effect of cheese type on external appearance (shape, crust), color, dough, cross-section, odour and taste parameters. Mean comparison was done by Duncan test (Duncan Multiple Range Test) where statistical significance was shown at the $P<0.05$ probability level.

RESULTS AND DISCUSSION

The result showed that, in general for all types of cheese from the research, taste is typical, unevently salty with mild milky aromas, and some examples revealing notes of bitterness. Consistency was soft, brittle, consistent with a medium hardness and the color ranged from white to pale yellow. When discussing form, the common shapes were rectangles or triangles and the smell is slightly sour and milky.

The best-rated type of cheeses is ewe's milk cheese, which got 18.45 points of a maximum of 20, as shown in Table 3. It is classified as cheese with excellent sensory properties, especially when it comes to tastes and cross-sectional appearance. The cheese made from mixed milk obtained 17.63 points, that, also, classified it to the group of excellent quality cheese, and scored an average taste rating of 8.88 indicates its excellent quality (table 2). The obtained points show that cow cheese is classified as good quality cheese. Cow's milk cheese received the lowest rating because of its poorer cut and consistency scores, but the listed flaws of the cheese are such that they are still acceptable, meaning that the samples had only minor structural flaws. Statistical analysis revealed that differences in all obtained parameters were not statistically significant.

Table 2. The results of the sensory evaluation of the quality of the Kučki cheese

Type of cheese	External appearance	Color	Consistency	Cross section	Odour	Taste
	2	1.9	1.6	1.6	1.5	8
	1.9	1.9	2	1.6	1.8	9
Cow	2	2	1	1.5	1.7	8
	2	2	1.6	1.7	1.5	8.5
$\overline{X}$ ± SD	**1.98±0.05**	**1.95±0.06**	**1.55±0.41**	**1.60±0.08**	**1.63±0.15**	**8.38±0.48**
	1.5	1.9	1.9	2	1.6	9
Mixed	1.6	1.9	1.5	1.6	1.7	8.5
	2	2	1	1.7	2	9
	2	1.9	1.5	1.7	2	9
$\overline{X}$ ± SD	**1.78±0.026**	**1.93±0.05**	**1.48±0.37**	**1.75±0.17**	**1.83±0.21**	**8.88±0.25**
	1.9	2	2	1.9	2	10
Ewe	2	1.6	1.5	2	2	8
	2	2	1	1.4	1.7	9
	2	2	2	2	1.8	10
$\overline{X}$ ± SD	**1.98±0.05**	**1.90±0.20**	**1.63±0.48**	**1.83±0.29**	**1.88±0.15**	**9.25±0.96**

The slice of cheese is similar in shape, firmness, and color to the Sjenički cheese results referred by Jovanović *et al.* (2004). In contrast, the saltiness of the Kučki cheese samples was quite uneven compared to the Feta type cheeses Tratnik *et al.* (2000), which had a lower salinity concentration.

The changes in the sensory properties are in agreement with those reported by Tratnik *et al*. (2000) for Domiati cheese, where as ripening progresses, the taste and smell intensify, which is similar to Kučki cheese. Kučki cheese has a sharper taste, in contrast to Golijski cheese, where Ostojić (2010) states that the taste resembles a young walnut. The consistency appears as an uneven triangle or rectangle and is soft, easily breakable, but not brittle. The cross section is closed or has cavities, and the color is white or yellow/white. Those characteristics of slices of Kučki cheese are similar with results of scientific paper whose topic was Pljevaljski cheese (Mirecki and Konatar, 2014). Stronger salting in Kučki cheese is also characteristic of Sozinski cheese, which is described, by Dozet *et al.*, (1996), as a round shape with firm structure, while it breaks a little when cut, preferably white to white-yellow. Travnički cheese produced from cow's milk also has a whitish-yellow color and a specific pleasant lactic acid taste (Dozet, 2004). Homoljski sheep's cheese, unlike Kučki, does not have a cavity on the cut, and also has a spicy and salty taste (Popović-Vranješ, 2015).

Table 3. Total results of the sensory evaluation of Kučki cheese

Cheese type	Evaluator 1.	Evaluator 2.	Evaluator 3.	Evaluator 4.	Results
Ewe	19.8	17.1	17.1	19.8	**18.45**
Mixed	17.9	16.8	17.7	18.1	**17.63**
Cow	16.6	18.2	16.2	17.3	**17.08**

The alterations in the sensory characteristics are consistent with those noted by Tratnik *et al.* (2000) for Domiati cheese, which is comparable to Kučki cheese in that as ripening advances, the flavor and odour intensify. Compared to Golijski cheese, which Ostojić *et al*. (2010) says tastes like a young walnut, Kučki cheese has a harsher flavor. The results of sensory analysis in table 3. showed that ewe's and mixed types of cheeses had excellent and cow's cheese had good quality.

Characteristics of slices of Kučki cheese are following: height: 2.0-4.0 cm; length: 10-15 cm; width: 5-10 cm. The consistency is soft, easily breakable, but not brittle with appearance of irregular triangle or rectangle.

Table 4. Quality categories of food products (Ritz *et al*., 1991)

Quality Category	Cheese type			
	Points scale	Cow	Mixed	Ewe
Excellent	17.6-20.0	-	17.63	18.45
Good	15.2-17.5	17.08	-	-
Mediocre	13.2-15.1	-	-	-
Still acceptable	11.2-13.1	-	-	-
Unacceptable	<11.2	-	-	-

CONCLUSIONS

Montenegro is known for a wide range of traditional dairy products, especially cheese. One of them is Kučki cheese, which belongs to the group of white brined full-fat cheeses. Production is organized in villages and on katuns. Although it is a nutritionally valuable product, in the last 30 years there has been no significant research on the Kučki cheese, which is an unavoidable segment in the history and culture of Kuči region. Indigenous dairy products have always had their consumers, today's requirements relate to quality with a safe geographical origin. According to the obtained results, ewe's milk cheese had the highest total points of all type of analyzed cheeses, scoring 18.45 out of a possible 20, which classified it as a excellent quality cheese with exceptional sensory qualities, particularly in terms of taste and cross-sectional appearance. Characteristics of slices of Kučki cheese are following: height: 2.0-4.0 cm; length: 10-15 cm; width: 5-10 cm. The consistency is soft, easily breakable, but not brittle with appearance of irregular triangle or rectangle. The color is white, yellow/white and the cross section is closed or with cavities.

Kučki cheese has a specific stronger, a little bitter taste, but typical for this traditional–autochthonous cheese type. In conclusion, Kučki cheese is a part of the cultural heritage of this area of Montenegro that should be protected, standarised and better valorised.

REFERENCES

Buchin, S., Delague, V., Duboz, G., Berdagué, J., Beuvier, E., Pochet, S., & Grappin, R., (1998). Influence of Pasteurization and Fat Composition of Milk on the Volatile Compounds and Flavor Characteristics of a Semi-hard Cheese. Journal of Dairy Science, 81, 3097-3108.

Carvalho, G.; Santos, R.; Fino, A.; Ferreira, P.; M. Rodrigues, F.; Dias, J., (2020). Evolution during Three Ripening Stages of Évora Cheese. Foods; 9, 1140. https://doi.org/10.3390/foods9091140

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

Dozet N., (2004). Travnički-vlašićki cheese – white brine cheeses (in Bosnian). editor prof. dr Natalija Dozet, Poljoprivredni fakultet Srpsko Sarajevo, 43.

Dozet, N., Adžić, N., Stanišić, M., Živić. N., (1996). Autohtoni mlječni proizvodi, Poljoprivredni institut-Podgorica, Silmir, Beograd.

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

El-Nimr, A.; Eissa, H.; El-Abd, M.; Mehriz, A.; Abbas, H.; Bayoumi, H., (2010). Water Activity, Color Characteristics and Sensory Properties of Egyptian Gouda Cheese during Ripening. J. Am. Sci., 6, 447–453.

Fatma A. M. Hassan, Mona A M. Abd El- Gawad, A. K. Enab., (2013). Flavour Compounds in Cheese (Review), Research on Precision Instrument and Machinery Vol. 2 Iss. 2, June 2013.

Gulzar N, Sameen A, Muhammad Aadil R, Sahar A, Rafiq S, Huma N, Nadeem M, Arshad R, Muqadas Saleem I., (2020). Descriptive Sensory Analysis of Pizza Cheese Made from Mozzarella and Semi-Ripened Cheddar Cheese Under Microwave and Conventional Cooking. Foods; 9(2):214. https://doi.org/10.3390/foods9020214

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

Jokanović., O, Marković., B, Miočinović., J, Mirecki, S., (2021). Technology and quality of Kučki cheese - traditional Montenegrin brined cheese. Journal of Hygienic Engineering and Design, Vol. 33, pp. 76-84.

Jovanović, S., Maćej, O., & Barać, M., (2004). Characteristics of autochthonous production of Sjenica cheese at Sjenica-Pester plateau region. in Biotechnology in Animal Husbandry Institut za stočarstvo, Beograd., 20(1-2), 131-139.

Marković, M., Radonjić, D., Đokić, M., Kandić, A., Marković, B., (2021). Allelic polymorphism of k-casein gene (csn3) in three Montenegrin cattle breeds. Agriculture and Forestry, 67 (3): 61-70.

Martínez S, Carballo J., (2021). Physicochemical, Sensory and Nutritional Properties of Foods Affected by Processing and Storage. Foods; 10(12):2970. https://doi.org/10.3390/foods10122970

Mendia, C.; Ibañez, F.; Torre, P.; Barcina, Y., (1999). Effect of pasteurization on the sensory characteristics of an ewe's-milk cheese. J. Sens. Stud. 1999, 14, 415–424.

Mirecki S., Konatar Z., (2014). Technology and quality of Pljevlja cheese-traditional Montenegrin dairy product, Journal of Hygienic Engineering and Design 2014 Vol.6 pp.208-214, ref.20.

Ostojić, M., Topisirović, Lj., Relić R, Ježa, G., (2010). Autohtona tehnologija proizvodnje golijskog sira, Preh. ind. - Mleko i ml. proiz. 1-2 (2010) 46-51.

Popović-Vranješ, A., (2015). Specijalno sirarstvo. Novi Sad: Univerzitet u Novom Sadu-Poljoprivredni fakultet-Departman za stočarstvo, pp. 403.

Ritz M, Vojnović V, Vahnčić N., (1991). Sistem bodovanja u senzornoj procjeni kvalitete sira, Mljekarstvo (5) 127-135.

Santa, D., Srbinovska, S., Simonovska, T., (2021). Dynamics of acidity (pH) and temperature during the production of traditional Macedonian Galichki kashkaval. Agriculture and Forestry, 67 (3): 99-107.

StatSoft. STATISTICA 12:2012, Copyright StatSoft (http://www.statsoft.com/

Tratnik Lj, Božanić R, Harjač A, Kozlek D., (2000). Optimiranje proizvodnje i kakvoće sireva u salamuri tipa Feta i Domiati, Mljekarstvo 50 (3) 227-238.

Živković S., (1971). Tehnologija belog srpskog sira, Institut za mlekarstvo SFRJ, Novi Beograd.

Solhi-Khajehmarjan, R., Ghassemi-Golezani, K. (2023): Foliar spray of salicylic acid improved yield parameters and essential oil production of dill under water deficit. Agriculture and Forestry, 69 (4): 253-263. doi:10.17707/AgricultForest.69.4.17

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FOLIAR SPRAY OF SALICYLIC ACID IMPROVED YIELD PARAMETERS AND ESSENTIAL OIL PRODUCTION OF DILL UNDER WATER DEFICIT

SUMMARY

Drought stress causes physiological disorders and growth restriction, leading to a decrease in crop productivity in the field. Foliar spray of salicylic acid may reduce the adverse effects of this stress on plant performance. Thus, this experiment was laid out as split plot with RCB design in three replicates to assess changes in morpho-physiological traits and essential oil production of dill (*Anethum graveolens* L.) seeds in response to irrigation intervals (water supply after 70, 100, 130, 160 mm evaporation) and foliar spray of water (control) and salicylic acid (0.6, 1.2 mM). The results showed that, plant height, plant biomass, seeds per umbel and plant were diminished due to water deficit, leading to a decline in seed yield. 1000-seed weight was slightly increased under stress, indicating the extent of drought tolerance of dill plants. Application of salicylic acid improved plant height, branches per plant, leaves per plant, umbels per plant, 1000-seed weight, plant biomass and finally seed yield under different irrigation intervals, especially under water limitation. Essential oil content and yield of seeds were increased with decreasing water supply up to moderate stress. The percentage and yield of essential oil were enhanced up to 54 days after flowering, and thereafter were slightly reduced. In general, application of 1.2 mM salicylic acid on non-stressed and drought-stressed plants was the superior treatment to improve field performance and essential oil production of dill. Therefore, drought tolerance and performance of plants can be promoted by salicylic acid treatment.

Keywords: dill; essential oil; harvest time; salicylic acid; seed yield; water stress

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INTRODUCTION

Medicinal plants are considered as valuable medicines in the world (Salmeron-Manzano *et al.,* 2020). Plants produce numerous secondary metabolites, known as allelochemicals that are involved in biochemical defense mechanisms. Most of them can be used as herbicide and pesticide (Yamada *et al.*, 2019). Plant Secondary metabolites are increasingly important factors in plant diversity and evolution (Mohammadi *et al.*, 2020). Essential oils are important secondary metabolites that are used worldwide to produce perfumes, cosmetics, drinks, medicines, fungicides, and insecticides (Chouhan *et al*., 2017).

Anethum graveolens known as dill is an annual herb of Umbels (*Apiaceae*) family, native to the Mediterranean and western Asia (Kharadi *et al*., 2019). The dill fruit has paired carpels (schizocarps), which are released at maturity (Ghassemi-Golezani *et al.*, 2016). Most of the dill essential oil is produced in seeds and flowers, although leaves and stems contain essential oil (Dimov *et al.*, 2019). The fruits of dill contain essential oil comprising of major compounds such as carvone (58 %), limonene (37%), α-phellandrene (36%), and limonene (31 %) in the leaves and in the seeds (Saıd-Al Ahl and Omer, 2016).

Studies have shown that essential oil of dill seeds has antiseptic, anti-carcinogenic, anti-hyperlipidemic properties, relieves intestinal spasms and griping, and helps to settle colic (Chahal *et al.*, 2017). Seed development is a series of events involving cell division, cell differentiation, and macromolecular storage. In cotyledons, cell differentiation begins in some parts and gradually spreads to other parts, thus building up a gradient for development. Seeds accumulate starch, store proteins, and oil in various proportions, depending on species and environmental conditions (Sagun *et al.,* 2023). Adverse environmental conditions may cause various responses in medicinal plants such as changes in growth and secondary metabolites (Isah, 2019). Major environmental stresses affecting crop performance are biotic or abiotic. Water shortage is the most important stress factor that limits crop growth and production via disruption of physiological and metabolic activities (Ahmad *et al.*, 2022). Reduction in cell turgor is the first sign of drought stress in many plants, which decreases plant growth and development. Plants tend to reduce transpiration under water shortage by decreasing the number and size of the leaves (Zhou *et al.*, 2021). Increasing drought stress significantly reduced the number of branches and seed yield in coriander plants. Water limitation during flowering and seed filling stages increased flower abortion, thus decreasing the seeds number per plant (Yeganehpoor *et al*., 2019). Reduction in seeds number per plant was also due to a decrement in flower formation (Benezit *et al.*, 2017). Drought stress during reproductive stages can decrease flowering and seed filling periods, that lowers the number and weight of seeds per plant (Jamshidi Zinab *et al.*, 2022). It was reported that irrigations after 130 and 160 mm evaporation caused a significant decrease in the yield components and seed yield of the safflower, compared to the control plants. While there was no significant difference between irrigations after 70 and 100 mm evaporation (Ghassemi Golezani *et al*., 2022). The essential oil percentage of plant organs increases with increasing water deficit during plant growth and development, but the essential oil yield decreases as a result of a large reduction in organs yield (Ghassemi-Golezani and Solhi-

Khajemarjan, 2021). Another well-adaptive response of plant species to drought stress is the rise in osmolytes such as soluble sugars and proline (Sharma *et al.*, 2019). The essential oil content of dill seeds may increase with decreasing water supply, leading to a highest essential oil yield in mild and moderate drought stresses (Ghassemi-Golezani *et al*., 2016).

Exogenous application of signaling molecules such as salicylic acid (SA) can reduce some of the detrimental impacts of drought stress on plants. Salicylic acid is an endogenous phenolic compound that regulates plant physiological and biochemical processes to improve growth, photosynthesis and productivity. This phytohormone modulates the syntheses of osmolytes and secondary metabolites to protect plants from adverse conditions. The SA induces enzymatic and non-enzymatic antioxidant activities in plants (Farhadi and Ghassemi-Golezani, 2020). A moderate concentration of SA may improve the antioxidant capacity of the plants under stress; but a higher rate of this hormone may limit plant growth (Liu *et al.,* 2022). Foliar application of SA enhances branches per plant and seed yield of coriander under different levels of water supply (Yeganehpoor *et al.*, 2019). This natural regulator causes a considerable increment in essential oils extracted from young shoots and peels of grapefruit (Khalid *et al*., 2018). Seed production of okra Plants treated with 1.2 mM salicylic acid was enhanced by 11.13%, compared to control plants (Rodrigues da Silva *et al*., 2023). Application of about 1.3 mM salicylic acid on tomato plants under drought stress reduced flower shedding and increased plant production by increasing gas exchange (Aires *et al*., 2022). Foliar application of 1 mM SA also increased photosynthetic pigments, plant biomass and essential oil production in Thymus vulgaris (Miri *et al*., 2015). Thus, this research was designed to examine changes in morphology, yield parameters, and essential oil production of dill plants in various levels of water stress and salicylic acid treatments.

MATERIAL AND METHODS

Location and experimental design

This research was conducted in 2019 at the Research Farm of the University of Tabriz, Iran, to evaluate essential oil accumulation in seeds of SA untreated (SA0: water spray) and treated (SA1: 0.6 and SA2: 1.2 mM) plants under different watering levels (I1, I2, I3, I4: irrigation after 70, 100, 130, 160 mm evaporation from a class A pan as normal irrigation and mild, moderate and severe stresses, respectively). The experimental work was performed as split-plot with randomized complete block design in three replications. Irrigation intervals and salicylic acid levels were allocated to main and sub plots, respectively. Each plot had six rows with a length of 5 m and a distance of 25 cm. Dill seeds (Tabriz ecotype) were treated with 2 g kg^{-1} Benomyl and then were seeded (80 seeds m^{-2}) at a depth of 1.5 cm of sandy loam soil. The plots were regularly irrigated from sowing up to seedling establishment, and subsequent irrigations were performed in accordance with the treatments. The weeds within the plots were removed at different stages of plant growth and development. The plants were sprayed by water (control) and SA at vegetative (48 days after sowing) and flowering (80 days after sowing) stages.

Measurements

At full flowering stage, 10 plants from each plot were harvested and plant height, leaves per plant, and branches per plant were determined.

At maturity, plants in 1 m^2 of each plot were harvested and number of umbels per plant, 1000-seed weight and seed yield were recorded. Then, seeds per umbel and seeds per plant were calculated. These plants were then dried in an oven at 80° C for 48 hours and above ground biomass was determined.

At each of six stages of seed development (26, 33, 40, 47, 54 and 61 days after flowering) 30 plants from each plot were harvested and the seeds were detached from plants. The seed samples were then dried at a room temperature of 20-25 ºC for 14 days. A sub-sample of 30 g powdered seeds from each plot was well mixed with 500 ml double-distilled water, and then the essential oil was extracted by hydro-distillation at 250 ºC for 3 h, using a Clevenger (Wang and Zhang, 2020). The essential oil percentage and yield were calculated as:

Essential oil percentage = (essential oil weight/seed weight) ×100

Essential oil yield (g m^{-2}) = essential oil percentage × seed yield (g m^{-2})

Statistical analysis

Analysis of variance of data was performed appropriate to experimental design, but essential oil accumulation at different stages of seed development was analyzed as split-split plot with randomized complete block design, using MSTAT-C software. The data means were compared following the Duncan multiple range test at $p \leq 0.05$. The figures were drawn by Excel software.

RESULTS AND DISCUSSION

Morphological traits and plant biomass

Data analysis showed (Table 1) that plant height and plant biomass were significantly affected by irrigation intervals and SA levels. Leaves and branches per plant were only affected by SA, and leaf water content was only influenced by water stress.

Table 1. Analysis of variance of the data for morphological traits of dill affected by water supply and salicylic acid

Treatments	df	Mean squares			
		Plant height	Branches per plant	Leaves per plant	Plant biomass
Replication	2	4.43	0.21	0.42	2102.90
Irrigation (I)	3	511.14**	6.90	2.07	51760.47**
Ea	6	5.33	5.57	1.25	2048.34
Salicylic acid (SA)	2	52.22**	7.23**	7.10**	36737.08**
I × SA	6	11.38	0.52	0.34	3592.46
Eb	16	4.25	0.83	0.23	1484.90
CV (%)	-	4.22	22.50	9.19	8.60

***, significant p ≤ 0.01, respectively*

The highest plant height and plant biomass were obtained under normal irrigation (I1) and decreased with increasing water stress (Table 2). The greatest improvement in plant biomass (about 23%), plant height (about 9%), branches per plant (about 52%) and leaves per plant (29%) was achieved by 1.2 mM SA, followed by 0.6 mM SA, although there was no significant difference between 0.6 mM treated and untreated plants.

Table 2. Average plant height, branches per plant and leaves per plant of dill under different irrigation treatments in response to salicylic acid

Treatments	Plant height (cm)	Branches per plant	Leaves per plant	Plant biomass (g m^{-2})
Irrigation (I)				
I_1	59.71±0.50 a	--	--	517.85±7.59 a
I_2	48.12±1.37 b	--	--	482.30±20.03 a
I_3	43.97±1.64 c	--	--	449.00±30.68 a
I_4	43.54±0.60 c	--	--	342.18±14.55 b
Salicylic acid (SA)				
SA_0	47.07±2.16 b	2.16±0.33 b	4.56±0.15 b	403.83±24.39 b
SA_1	48.30±2.04 b	2.59±0.47 ab	5.10±0.25 b	429.72±27.70 b
SA_2	51.14±2.16 a	3.67±0.41 a	6.10±0.34 a	509.95±26.09 a

Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test)

Yield components and seed yield

Irrigation intervals and salicylic acid levels showed significant effects on umbels per plant, seeds per umbel, 1000 seed weight and seed yield of dill (Table 3). The seeds per plant was only affected by irrigation treatment. The interaction of water stress × salicylic acid was not significant for all these traits.

Table 3. Analysis of variance of the effects of water stress and salicylic acid on yield components and seed yield

Treatments	df	Mean squares				
		umbels per plant	Seeds per umbel	Seeds per plant	1000 Seed weight	Seed yield
Replication	2	0.61	1461.88	109902.43	1.83	92.57
Irrigation (I)	3	3.52*	168947.05**	2641140.84**	21.17**	18691.22**
Ea	6	0.45	2084.26	35360.50	0.41	335.27
Salicylic acid (SA)	2	4.06**	28993.26**	55199.89	2.88**	8759.20**
I × SA	6	0.50	1416.12	31717.12	0.15	582.54
Eb	16	0.30	2740.23	26234.52	0.09	218.36
CV (%)	-	13.25	20.34	16.01	9.61	7.01

**, **: significant at p ≤ 0.05 and p ≤ 0.01, respectively*

The mean number of umbels per plant (about 21%) was significantly decreased under severe water deficit, but no significant decline of this traits was observed under mild and moderate drought stresses (Table 4). The seeds per umbel (about 39%) and per plant (about 61%) were significantly reduced under all limited irrigation, compared to normal irrigation. No significant differences were observed in umbels per plant between mild and moderate stresses and in seeds per plant among all stress treatments. The largest seeds were produced under mild stress, which had no significant difference with moderate stress. The 1000 seeds weight was statistically similar for normal irrigation and severe stress. Seed yield per unit area (up to 51%) was significantly decreased under moderate and severe stresses. However, there was no significant difference between normal irrigation and mild stress. Foliar spray of salicylic acid, particularly with 1.2 mM concentration, significantly enhanced, umbels per plant (about 28%), seeds per umbel (about 35%), 1000 seed weight (about 31%) and seed yield per unit area (about 24%).

Table 4. Means of plant biomass, yield components and seed yield of dill affected by irrigation intervals and salicylic acid levels

Treatments	Umbels per plant	Seeds per umbel	Seeds per plant	1000 seed weight ($g\ m^{-2}$)	Seed yield ($g\ m^{-2}$)
Irrigation (I)					
I_1	4.14±0.28 ab	435.87±35.60 a	1794.20±131.01 a	1.76±0.05 b	248.21±4.70 a
I_2	4.14±0.99 ab	160.19±29.40 c	633.32± 127.24 b	4.86±0.33 a	237.56±10.12 ab
I_3	4.90±0.95 a	140.20±24.23 c	662.54±97.38 b	4.04±0.24 a	211.21±14.65 b
I_4	3.36±0.69 b	293.17±35.89 b	957.04± 158.95 b	1.97±0.14 b	146.64±12.64 c
Salicylic acid (SA)					
SA_0	3.52±0.23 b	219.54±49.09 b	--	2.68±0.35 c	189.50±14.44 b
SA_1	4.20±0.32 a	239.61±60.49 b	--	3.13±0.46 b	201.94±11.09 b
SA_2	4.68±0.27 a	312.92±63.43 a	--	3.66±0.47 a	241.26±7.63 a

Essential oil accumulation and yield

Essential oil percentage and yield of dill seeds were significantly affected by irrigation intervals, salicylic acid levels and harvest time (Table 5). The interaction of irrigation × harvest time was also significant for both traits.

Table 5. Analysis of variance of the data for essential oil percentage and yield of dill seeds affected by irrigation intervals and salicylic acid levels

Treatments	df	Mean squares	
		Essential oil percentage	Essential oil yield
Replication	2	0.40	2107.01
Irrigation (I)	3	1.62*	5489.21**
Error (I)	6	0.16	392.25
Salicylic acid (SA)	2	3.17**	6678.78**
I × SA	6	0.05	328.47
Error (SA)	16	0.02	132.96
Harvest time (T)	5	3.11**	33413.63**
I × T	15	0.17**	944.52**
SA × T	10	0.03	434.02
I × SA × T	30	0.01	325.52
Error (T)	120	0.06	236.41
CV (%)	-	19.80	21.98

*, ** *significant at $p \leq 0.05$ and $p \leq 0.01$, respectively*

The highest essential oil percentage and yield were recorded under mild stress, followed by moderate stress (Table 6). There was no significant difference in essential oil percentage among different levels of stress. However, essential oil yield under severe stress was lower than other stress levels. Salicylic acid enhanced essential oil percentage (up to 32%) and yield (up to 25%), but to improve the essential oil percentage of dill seeds, 1.2 mM SA was the superior treatment.

Table 6. Means of essential oil percentage and yield for irrigation intervals and salicylic acid levels

Treatments	Essential oil percentage	Essential oil yield
Irrigation (I)		
I_1	1.01±0.02 b	19.59±1.45 b
I_2	1.37±0.02 a	25.80±2.01 a
I_3	1.38±0.05 a	23.76±1.42 ab
I_4	1.33±0.03 a	19.31±1.75 b
Salicylic acid (SA)		
SA_0	1.09±0.03 c	19.95±2.87 b
SA_1	1.23±0.09 b	20.80±2.67 b
SA_2	1.50±0.17 a	25.60±2.86 a

Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test)

With increasing seed development, the essential oil percentage of dill seeds was increased up to 54 days after flowering, and thereafter no tangible changes were observed. The essential oil content of seeds at all harvest times under normal irrigation was lower than stress levels. The highest essential oil percentage at final harvest was recorded under moderate stress (Figure 1A).

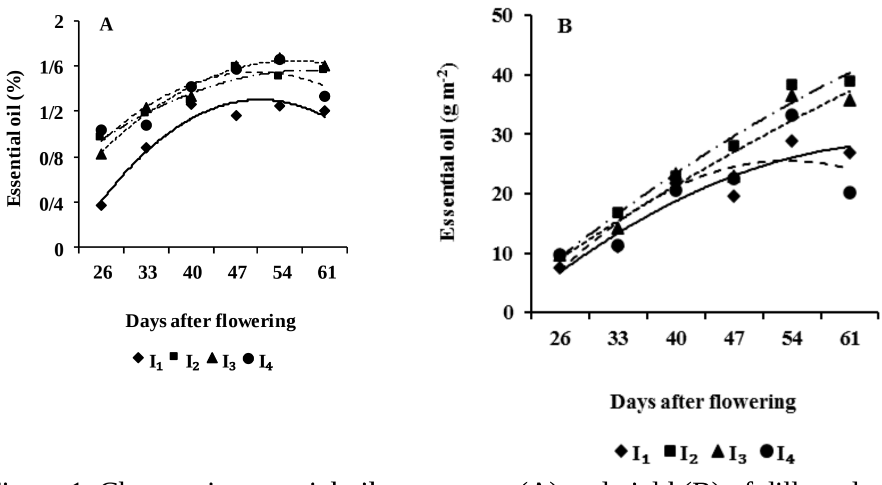

Figure 1. Changes in essential oil percentage (A) and yield (B) of dill seeds during development in response to irrigation

In contrast, essential oil yield per unit area under normal irrigation and mild and moderate stress levels was enhanced up to final harvest (61 days after flowering). However, under severe stress it was increased up to 54 days after flowering and after that it was slightly decreased. At the early stages of seed development, there was a little difference in essential oil production among irrigation intervals, but these differences were enhanced at subsequent harvests, particularly at seed maturity stage. The highest essential oil yield of dill seeds at this stage was obtained under mild stress, followed by moderate stress (Figure 1B).

CONCLUSIONS

Increasing irrigation intervals was led to a decrease in plant height, plant biomass and consequently seed yield in dill plants. However, the highest essential oil percentage and yield was recorded under mild and moderate water deficits. Foliar spray of SA, particularly with 1.2 mM concentration decreased the adverse effects of water limitation and enhanced essential oil percentage and yield via improving plant growth, seed weight and yield. Therefore, this could be introduced as an effective treatment for improving field performance and essential oil production of dill plants under various levels of water stress. Future investigation on the impacts of drought and SA levels on different medicinal plants may support the findings of this research.

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REFERENCES

Ahmad, S., Belwal, V., Punia, S.S, Ram, M., Rajput, S.S., Kunwar, R., Meena, M.K., Gupta, D., Kumawat, G.L., Hussain, T., & Mohamed, H.I. (2022). Role of plant secondary metabolites and phytohormones in drought tolerance: A Review. Gesunde Pflanzen. 1-18.

Aires, E.S., Ferraz, A.K.L., Carvalho, B.L., Teixeira, F.P., Putti, F.F., de Souza, E.P., Rodrigues, J.D., & Ono, E.O. (2022). Foliar application of salicylic acid to mitigate water stress in tomato. Plants. 11: 1775.

Al Hassan, M., Chaura, J., Donat-Torres, M.P., Boscaiu, M., & Vicente, O. (2017). Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. AoB Plants. 9: plx009.

Ali, B. (2021). Salicylic acid: An efficient elicitor of secondary metabolite production in plants. Biocatalysis and Agricultural Biotechnology. 31: 101884.

Arnao, M.B., & Hernández-Ruiz, J. (2018). Melatonin and its relationship to plant hormones. Annals of Botany. 121: 195-207.

Benezit, M., Biarnes, V., Jeuffroy, & M.H. (2017). Impact of climate and diseases on pea yields: what perspectives with climate change? Oleagineux, Corps Gras, Lipides. 24: D103.

Chahal, K., Dhaiwal, K., Kumar, A., Kataria, D., & Singla, N. (2017). Chemical composition of *Trachyspermum ammi* L. and its biological properties: A review. Journal of Pharmacognosy and Phytochemistry. 6: 131-140.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oil present status and future perspectives. Medicines. 4: 58.

Dimov, M., Dobreva, K., & Stoyanova, A. (2019). Chemical composition of the dill essential oils (*Anethum graveolens* L.) from Bulgaria. Bulgarian Chemical Communications. 51: 214-216.

Dullah, I.C., Sulandjari, S., & Supriyono, S. (2019). Abiotic stress and biofertilizer on the Pereskia bleo (Kunth) DC. Against growth, proline, and antioxidants. SAINS TANAH-Journal of Soil Science and Agroclimatology. 16: 36-45.

Duran, M.D.L., Zabala, M.E.A., & Londono, G.A.C. (2021). Optimization of flavonoid production in plant cell culture of *Thevetia peruviana* elicited with methyl jasmonate and salicylic acid. Brazilian Archives of Biology and Technology. 64.

Farhadi, N., & Ghassemi-Golezani, K. (2020). Physiological changes of *Mentha pulegium* in response to exogenous salicylic acid under salinity. Scientia Horticulturae. 267: 109325.

Ghassemi-Golezani, K., Ardalan, N., Raei, Y., & Dalil, B. (2022). Improving some physiological and yield parameters of safflower by foliar sprays of Fe and Zn under drought stress. Journal of Plant Physiology and Breeding. 12: 15-27.

Ghassemi-Golezani, K., Hassanzadeh, N., Shakiba, M.R., & Dalil, B. (2023). How Can Salicylic Acid and 24-epibrassinolide Improve Productivity and Oil Quantity and Quality of Black Mustard Under Salt Stress?. Gesunde Pflanzen. 75: 939-946.

Ghassemi-Golezani, K., Nikpour-Rashidabad, N. & Samea-Andabjadid, S. (2022). Application of growth promoting hormones alters the composition and antioxidant potential of dill essential oil under salt stress. Scientific Reports. 12: 14349.

Ghassemi-Golezani, K., Rezaeipour, L., & Alizadeh-Salteh, S. (2016). Effects of water stress on seed yield and essential oil content of dill genotypes. Journal of Biodiversity and Environmental Sciences. 9: 420-425.

Ghassemi-Golezani, K., & Solhi-Khajemarjan, R. (2021). Changes in growth and essential oil content of dill (*Anethum graveolens*) organs under drought stress in response to salicylic acid. Journal of Plant Physiology and Breeding. 11: 33-47.

Giannoulis, K., Platis, I., Gougoulias, N., & Wogiatzi, E. (2020). Influence of *Trichoderma harzianum* and *Glomus mycorrhiza* on biomass and essential oil yield of organic *Ocimum basilicum* cultivation. Agriculture & Forestry. 66: 139-154.

Huot, B., Yao, J., Montgomery, B.L., & He, S.Y. (2014). Growth defense tradeoffs in plants: a balancing act to optimize fitness. Molecular plant. 7: 1267-1287.

Ibrahim, M.H., Jaafar, H.Z., Karimi, E., & Ghasemzadeh, A. (2014). Allocation of secondary metabolites, photosynthetic capacity, and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) in response to and light intensity. The Scientific World Journal. 2014.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. Biological research. 52: 1-25.

Issa Ali, O., Fghire, R., Anaya, F., Benlhabib, O., & Wahbi, S. (2019). Physiological and morphological responses of two quinoa cultivars (*Chenopodium quinoa* Willd.) to drought stress. Gesunde Pflanzen. 71: 123–133.

Jamshidi Zinab, A., Hasanloo, T., Naji, A.M., Delangiz, N., Farhangi-Abriz, S., Asgari Lajayer, B., Hemati, A., Shobbar, Z.S., & Farooq, M. (2022). Physiological and biochemical evaluation of commercial oilseed rape (*Brassica Napus* L.) cultivars under drought stress. Gesunde Pflanzen. 1-14.

Khalid, K.A., El-Gohary, A.E., & Ahmed, A.M. (2018). Effect of the interaction between salicylic acid and geographical locations on grapefruit essential oil. Journal of Essential Oil-Bearing Plants. 21: 1594-1603.

Kharadi, M.K., Yadav, V.K., & Kumar, A. (2019). A review on traditional uses and therapeutic indications of *Anethum sowa*. World Journal of Pharmaceutical Research. 8: 239-248.

Kheloufi, A., Chorfi, A., Mansouri, L.M., & Benyamina, H. (2018). Morpho-physiological characterization and photosynthetic pigment contents of acacia Karroo Hayne seedlings under saline conditions. Agriculture & Forestry. 64: 87-99

Li, A., Sun, X., & Liu, L. (2022): Action of salicylic acid on plant growth. Frontiers in Plant Science. 13: 878076.

Liu, J., Qiu, G., Liu, C., Li, H., Chen, X., Fu, Q., Lin, Y., & Guo, B. (2022). Salicylic acid, a multifaceted hormone, combats abiotic stresses in plants. Life. 12: 886.

Lotfi, R., & Ghassemi-Golezani, K. (2015): Influence of salicylic acid and silicon on seed development and quality of mung bean under salt stress. Seed Science and Technology. 43: 52-61.

Ma, X., Zheng, J., Zhang, X., Hu, Q., & Qian, R. (2017). Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superb* us (*Caryophyllaceae*) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. Frontiers in Plant Science. 8: 600.

Mimouni, H., Wasti, S., Manaa, A., Gharbi, E., Chalh, A., Vandoorne, B., Lutts, S., & Ahmed, H.B. (2016). Does salicylic acid (SA) improve tolerance to salt stress in plants? A study of SA effects on tomato plant growth, water dynamics, photosynthesis, and biochemical parameters. Omics: a journal of integrative biology. 20: 180-190.

Miri, S.M., Ahmadi, S., & Moradi, P. (2015). Influence of salicylic acid and citric acid on the growth, biochemical characteristics and essential oil content of thyme (*Thymus vulgaris* L.). Journal of Medicinal Plants and By-products. 4: 141-146.

Mohammadi, H., Akhondzadeh, M., & Hatami, M. (2021). Exogenously applied 24-epibrassinolide modulates physiological and biochemical constituents in lavender (*Lavandula angustifolia*) plants under drought stress conditions. Agriculture & Forestry. 67: 103-120.

Mohammadi, H., Nikjoyan, J. M., Hazrati, S., & Hashempour, H. (2020). Improvement of yield and phytochemical compounds of Thymus vulgaris through foliar application of salicylic acid under water stress. Agriculture and Forestry. 66: 129-142.

Moustakas, M., Sperdouli, I., Adamakis, I.D.S., Moustaka, J., Isgoren, S., & Sas, B. (2022). Harnessing the role of foliar applied salicylic acid in decreasing chlorophyll content to reassess photosystem II photoprotection in crop plants. International Journal of Molecular Sciences. 23: 7038.

Oguz, M.C., Aycan, M., Oguz, E., Poyraz, I., & Yildiz, M. (2022). Drought stress tolerance in plants: Interplay of molecular, biochemical and physiological responses in important development stages. Physiologia. 2: 180-197.

Rering, C.C., Franco, J.G., Yeater, K.M., & Mallinger, R.E. (2020). Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. Ecosphere. 11: e03254.

Rodrigues da Silva, A.A., Francisco do Nascimento Sousa, P., Soares de Lima, G., Soares LAdA, Gheyi, H.R., de Andrade Silva, L., Rocha, J.L.A., & Azevedo, C.A.V. (2023). Salicylic acid alleviates the effects of salt stress on the physiology, growth, and production of hydroponic okra. Arid Land Research and Management. 1-17.

Sagun, J.V., Yadav, U.P., & Alonso, A.P. (2023). Progress in understanding and improving oil content and quality in seeds. Frontiers in Plant Science. 14: 105.

Said-Al Ahl, H., & Omer, E. (2016). Essential oil content and chemical composition of eight dill (*Anethum graveolens* L.) cultivars cultivated under Egyptian conditions. International Journal of Pharmacy and Pharmaceutical Sciences. 8: 227-231.

Salmeron-Manzano, E., Garrido-Cardenas, J.A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. International journal of environmental research and public health. 17: 3376.

Sehgal, A., Sita, K., Siddique, K.H., Kumar, R., Bhogireddy, S., Varshney, R.K., Hanumantha Rao, B., Nair, R.M., Prasad, P.V., & Nayyar, H. (2018). Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. Frontiers in Plant Science. 9: 1705.

Sharma, A., Shahzad, B., Kumar, V., Kohli, S.K., Sidhu, G.P.S., Bali, A.S., Handa, N., Kapoor, D., Bhardwaj, R., & Zheng, B. (2019). Phytohormones regulate accumulation of osmolytes under abiotic stress. Biomolecules. 9: 285.

Wang, Y.H., & Zhang, Y.R. (2020). Variations in compositions and antioxidant activities of essential oils from leaves of Luodian Blumea balsamifera from different harvest times in China. PLoS One. 15: e0234661.

Yamada, T., Hamada, M., Floreancig, P., & Nakabachi, A. (2019). Diaphorin, a polyketide synthesized by an intracellular symbiont of the Asian citrus psyllid, is potentially harmful for biological control agents. PLoS One. 14: e0216319.

Yeganehpoor, F., Shafagh-Kolvanagh, J., Ghassemi-Golezani, K., & Dastborhan, S. (2019). Can application of nitrogen fertilizers and salicylic acid improve growth and fruit yield of coriander under water deficit? Acta Scientiarum Polonorum Hortorum Cultus. 18: 87–97.

Zhou, H., Zhou, G., He, Q., Zhou, L., Ji, Y., & Lv, X. (2021). Capability of leaf water content and its threshold values in reflection of soil–plant water status in maize during prolonged drought. Ecological Indicators. 124: 107395.

Velimirović, A., Jovović, Z., Berjan, S., El Bilali, H. (2023): Consumer perceptions and market potential for reintroduction of traditional wheat varieties in Montenegro. Agriculture and Forestry, 69 (4): 265-274. doi:10.17707/AgricultForest.69.4.18

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CONSUMER PERCEPTIONS AND MARKET POTENTIAL FOR REINTRODUCTION OF TRADITIONAL WHEAT VARIETIES IN MONTENEGRO

SUMMARY

Local and organic food purchase is frequently associated with consumer motivation to protect environmental, animal, and human health and support local communities and cultural traditions. Crop genetic resources, particularly traditional varieties, have been significantly eroded by the introduction of modern, high-yielding varieties in the last half of the 20th century. This was particularly relevant for staple crops, which include wheat. Omitting the understanding of why the genetic erosion of agricultural crops occurred, through this study we gave an insight into consumer preferences for traditional products and local wheat varieties. The random survey examined 1178 different profiles and their consuming habits with the aim of assessing their preferences and the possibility of reintroduction of wheat landraces in Montenegro. Public opinion polls confirmed the negative perception on genetically modified organisms (GMOs), pesticides, and intensive production, as 90.2% of the consumers would prefer to have their own production, consider as safer alternative compared to conventional production. It is promising that 94.5% of the respondents would rather buy bread produced from a local wheat variety than from a modern, imported variety, while 88.8% of the respondents would pay a higher price for flour, bread, and other products from local varieties. If engaged in agricultural production, respondents rated production using traditional plant varieties very positively, aiming to produce authentic, local products (78.5%). Such attitudes open the possibility of reintroduction of traditional wheat varieties on the market

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and encourage policy revision in order to pay more attention to traditional wheat varieties.

Keywords: wheat, traditional varieties, traditional products, consumers, survey

INTRODUCTION

The choice of food is fundamentally determined by social and cultural influences that lead to differences in the usual consumption of certain foods (Sobal 2009, Chen and Antonelli 2020). These influences are subject to changes accompanied by improving living standards, lifestyle changes and market offers (Jevšnik *et al.,* 2008). Many studies indicate the risks of excessive use of pesticides and pest resistance, genetically modified organisms (GMOs), food-borne diseases, and environmental pollution, to which a certain number of consumers attach importance, and, for these reasons, they increasingly turn to healthy and safe food with specific nutritional requirements (Paull, 2015, Wunderlich *et al.,* 2015; Botelho *et al.,* 2016, Bilali *et al*., 2020).

Landraces are dynamic populations of agricultural plants characterized by historical origin and traditional cultivation systems (Carrosio, 2005, Jovovic *et al.*, 2011, Velimirović *et al.*, 2023). Their production is maintained by continuous multiplication through generations in a given set of climatic, soil and agricultural conditions (Villa *et al.* 2005, Zeven 1998). Such linkage between landraces and the environment enabled the development of specific phenotypic and adaptive characteristics, and in the last decades, landraces received more attention as potential sources for breeding (Starr *et al*. 2015, Fratianni *et al.* 2020). They are often linked to specific and unique organoleptic characteristics preferred by the consumers (Frankin *et al*., 2023).

The reintroduction of traditional varieties has gained considerable attention in the search for healthy, safe, sustainable, and culturally meaningful food options (Johansson *et al.,* 2021). Traditional crop varieties, including wheat traditional varieties, are addressing the growing consumer demand for products that not only satisfy nutritional needs but also resonate with cultural heritage and sustainable practices (Dwivedi *et al.,* 2016, Doncic *et al.,* 2019, Orsini *et al.* 2020). Montenegro, as a country of rich agricultural tradition and biodiversity, with a growing market demand for healthy food choices, stands as an ideal candidate to explore the potential of traditional wheat varieties in meeting these demands.

The aim of this scientific paper is to understand the consumers' perception regarding traditional wheat varieties and to assess the level of interest in their increased utilization. The expansion of internet users has notably enhanced the accessibility of vast pool of potential survey participants, facilitating the implementation of diverse online evaluations (Sills *et al*., 2002). Use of this methodology for market analysis can give valuable insights into consumers' willingness to pay premium prices for conventional products, as well as their curiosity regarding novel products sourced from genetic resources (Conto *et al.*, 2016). Thus, this study employs an online survey to engage with the Montenegrin population, dealing with their preferences, attitudes, and expectations concerning traditional wheat varieties and derived products. Our hypothesis posits that, in light of the growing emphasis on health-conscious dietary choices, there exists significant untapped potential in Montenegro for the production and trade of products derived from traditional wheat varieties. While comprehensive demographic and preference data were collected, limited insight of the interplay between demographic factors and consumer preferences, such as observed age, education, and occupation-based differences was obtained. These interactions can elucidate the dynamics shaping consumer choices and provide more targeted insights for food producers, marketers, and policymakers aiming to align their strategies with the diverse demands of consumers.

MATERIAL AND METHODS

A voluntary self-administered online survey was designed using the online survey platform Survio to facilitate distribution and data collection. It was conducted in the period from February 24 to April 4, 2020, targeting the population residing in Montenegro. A non-probability convenience sampling method was employed to disseminate the survey through various online channels, including social media, email, and relevant websites.

The survey questionnaire contained a variety of question types, including multiple-choice questions, Likert scale questions, and open-ended questions. The questionnaire was divided into three parts: an introductory part with demographic information, a part related to the attitude toward conventional and genetically modified food, and a third part related to traditional products and varieties.

Quantitative data from multiple-choice and Likert scale questions were analysed using the Statistical Package for Social Sciences (SPSS), while qualitative data from open-ended questions were subjected to content analysis.

RESULTS AND DISCUSSION

Out of a total of 3014 visits, 1178 questionnaires were successfully completed, resulting in an overall completion rate of 39.1%. This completion rate indicates a moderately successful engagement with the survey respondents. Females represented a notable majority of the respondents, accounting for 60.61% of the total, while males make up 39.39% (Figure 1). The age distribution was relatively balanced across the various age groups. The largest age group falls within the 35-44 category, constituting nearly 30% of the respondents. This is followed by the 25-34 age group at 28.10% and the 18-24 age group at 23.43%. Older age groups, 45-54 and 55 and over, have fewer participants at 13.92% and 5.18%, respectively. Occupation indicates the economic status of respondents, and it is an important driver in purchase decision-making (Puddephatt *et al*., 2020). The occupation data indicates that the majority of the respondents are engaged in full-time or part-time jobs, representing 69.86% of the total. Students make up 16.21% of the participants, while 11.38% report being unemployed. A smaller percentage, 2.55%, identifies as retired individuals. The majority of the respondents have attained at least a high school education (57.30% have completed university, and 29.50% have finished high school). A smaller portion holds advanced degrees, with 11.60% reporting a Master's or Doctor of Science. A very small percentage, 0.80%, reported having either no formal education or only elementary school education.

Food safety concerns and values attributed to organic food affect positively the attitude towards organic food (Lazaroiu *et al.*, 2019, Le- Anh and Nguyen- To 2020). When making food choices between two extremes: “I prefer to buy food produced traditionally or organically, with minimal use of artificial fertilizers and pesticides” marked with one and “I prefer to buy food from conventional production/food produced with the use of artificial fertilizers and pesticides” marked with five on a Likert scale, 43.5% of the respondents chose traditional production methods, 27.1% moderately preferred these products, and 23.3% were neutral. Food from conventional production was chosen by only 1.4%, and 4.8% of the respondents showed a moderate tendency to buy these products (Table 1). The average rating of respondents' preferences was 1.93, which indicates that the majority prefers to buy food produced in a traditional way or organic food compared to food from conventional production (Lazaroiu, *et al*., 2019).

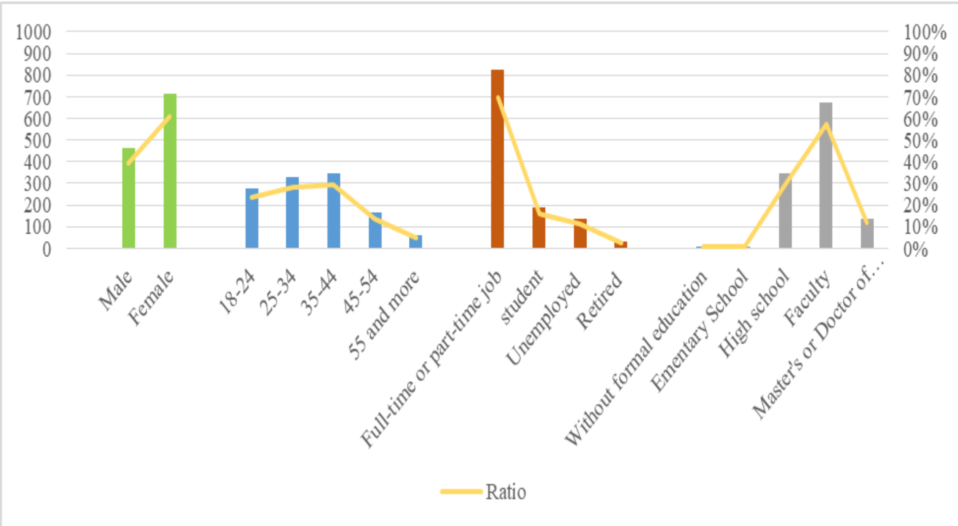

Figure 1. Profile of the respondents
Source: Survey, 2020

Education has a strong effect on the likelihood of buying organic products, as well as income (Dimitri and Dettmann 2012). Our findings indicate that the average score when choosing food is the highest among respondents aged 18-24, while it is the lowest among respondents over 55 years old (1.73 on a scale from 1 to 5). Respondents with a higher level of education gave lower ratings compared to those with a lower level of education. Slight differences are observed between the unemployed, who gave the highest average score (2.16), and pensioners (1.86), who prefer to buy food produced in a traditional way or organic food (Figure 2).

Table 1. Ratings of respondents' preferences when choosing food

	1	2	3	4	5	
I prefer to buy food produced traditionally or organically, with minimal use of artificial fertilizers and pesticides	512 43.5%	319 27.1%	274 23.3%	56 4.8%	17 1.4%	I prefer to buy food from conventional production/food produced with the use of artificial fertilizers and pesticides

Source: Survey, 2020

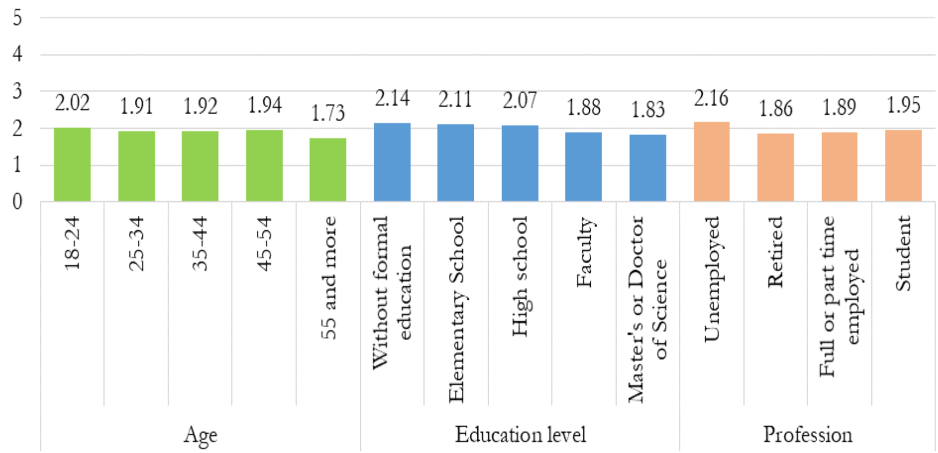

Figure 2. Average ratings of respondents' preferences in different categories when choosing traditionally produced/organic food and food from conventional production; Source: Survey, 2020

Consumers often associate better flavour and taste with old plant varieties of numerous crops found on the market (Sinesio *et al.*, 2021). Indeed, there is an increased interest in breeding communities to improve the sensory characteristics of modern varieties using traditional ones. Respondents who once consumed products from local varieties, singled out the taste as an important characteristic of this group of products in the largest percentage (79%), while slightly more than half of the respondents (56%) marked smell as a special characteristic. The non-sensory attributes of food products, such as health benefits, are often associated with organic and traditional food (Ditlevsen *et al.*, 2019). Every second respondent marked the nutrition value of the product as a special characteristic, while 44.5% of the respondents see health benefits (treatment of diseases, traditional recipes for colds, digestive diseases, etc.) as a special characteristic of products produced from local varieties (Figure 3).

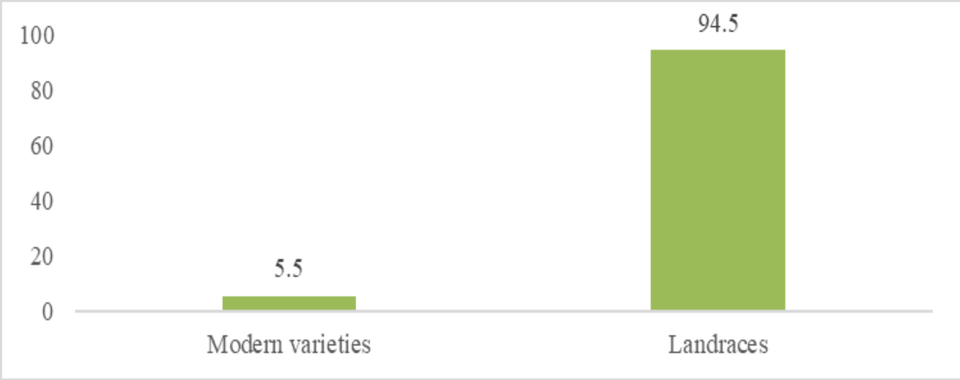

Figure 3. Distinctive properties of products from local varieties.
Source: Survey, 2020

Men more often indicated taste and smell as special characteristics of products from local varieties compared to women (81.3% and 59.4% vs. 77.5% and 53.7%), while women gave greater priority to nutrition and health benefits (52% and 47% versus 47.5% and 40.6%).

Traditional food products are an integral part of culture, identity, and heritage (Guerrero *et al*., 2009). European consumers are willing to exchange the higher cost and longer preparation time associated with traditional food in favour of the distinctive taste, quality, visual appeal, nutritional value, health benefits, and safety offered by traditional food products (Almli *et al.*, 2011). Of the total number of respondents, as many as 94.5% stated that they would rather buy bread made from a local variety of wheat than from a modern, imported variety. Observed by the level of education, respondents with lower education gave greater importance to modern, imported varieties of wheat compared to those with higher education. Of the total .number of respondents without formal education, 28.6% answered that they would rather buy bread from modern imported varieties of wheat compared to local varieties, while the percentage of respondents with completed elementary school was at the level of 22.2%. The number of respondents with higher education who would prefer to buy bread made from modern imported wheat varieties was significantly lower (4.7% of respondents with a university degree and 2.4% of the respondents with a master's degree and/or doctorate). There are differences in the answers observed from the point of view of work engagement. Of the total number of unemployed respondents, 88.4% would prefer to buy bread produced from a local variety of wheat, while a slightly lower percentage was recorded among the group of pensioners (90.5%). Out of the total number of employees, 96.3% would rather buy bread from the local wheat variety.

The smallest percentage of respondents would pay 75% more for flour, bread and other products from local varieties than the price of conventional products, while 11.2% are not ready to allocate a larger amount of money for their purchase. As much as 18.9% of respondents would pay twice the price, 31.2% would pay 25%

more, and 34.4% would be willing to pay 50% more than the price of conventional products (Figure 4).

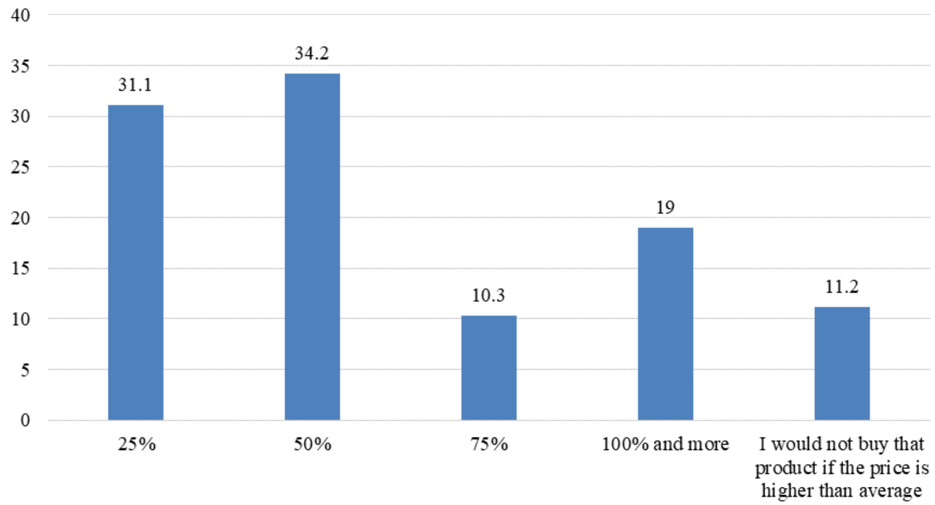

Figure 4. Willingness of the respondents to pay higher price for bread produced from traditional wheat variety; Source: Survey, 2020

Out of the total number of respondents who would not buy a product whose price is higher than the average, respondents older than 45 have a larger share. Also, this attitude is significantly more present among pensioners (33.3%), compared to other categories of the population. Although the majority of consumers consider traditional food products cheaper, the willingness to pay higher prices is confirmed throughout diverse markets (Pieniak *et al.*, 2009, Stolz *et al.*, 2011).

CONCLUSIONS

Understanding consumers' demands is essential for food producers, marketers, and policymakers to align their strategies and promote traditional and local food options. Our results suggest a notable preference toward organically and traditionally produced foods overall. The main determinants for such choices revealed in our study are occupation, education and age. Occupation is crucial in purchase decision-making, suggesting that the economic status of respondents plays a significant role in shaping their food choices. Education levels influenced food preferences, with respondents having lower levels of education expressing a higher preference for traditional or organic food. Conversely, those with higher education levels tend to have lower ratings for these preferences. Younger respondents (aged 18-24) express the highest average preference score for traditional and organic food, while older respondents over 55 show the lowest preference. Taste and smell and health benefits are highlighted as crucial characteristics of products from local varieties. The sensory traits, preferred by the male population, and health benefits preferred by females, are important findings for market-oriented production and appeal to the producers. A substantial majority of respondents express a strong preference and willingness to pay more for bread made from a local variety of wheat over modern, imported varieties, reflecting a strong connection to locally sourced products and highlighting the potential for supporting local agriculture.

While this study provides valuable insights into consumer preferences for traditional and organic food products, there are certain limitations that should be considered in future research. The predominantly female and relatively younger respondent profile could introduce bias. In-depth interviews or focus group discussions, could uncover deeper insights into the underlying motivations driving consumer preferences for traditional and organic food. Thus, future research will strengthen the applicability of the complex interplay between demographics and food choices.

REFERENCES

Almli, V. L., Verbeke, W., Vanhonacker, F., Næs, T. & Hersleth, M. (2011). General image and attribute perceptions of traditional food in six European countries. Food quality and preference, 22(1), 129-138.

El Bılalı, H., Bassole, I. H. N., Dambo, L., Berjan, S. (2020). Climate change and food security. Agriculture and Forestry, 66 (3): 197-210

Botelho, A., Dinis, I. & Lourenco Gomes, L. (2016). Understanding Consumer Preferences for Traditional Varieties of Apples in Portugal. Conference: ESADR 2016. Volume: Políticas Públicas para a Agricultura pós 2020. September 7–9, 2020. Coimbra, Portugal. Book of Proceedings.

Carrosio, G. (2005). Traditional Local Varieties between Traditions and Sustainable Agriculture. XXI Congress of the European Society for Rural Sociology. August 21–26. Keszthely, Hungary. Book of Proceedings.

Chen, P. J. & Antonelli, M. (2020). Conceptual models of food choice: influential factors related to foods, individual differences, and society. Foods, 9(12), 1898.

Conto, F., Antonazzo, A. P., Conte, A. & Cafarelli, B. (2016). Consumers Perception of Traditional Sustainable Food: An Exploratory Study on Pasta Made from Native Ancient Durum Wheat Varieties. Rivista di Economia Agraria, 71 (1): 325–337.

Dimitri, C. & Dettmann, R.L. (2012). Organic Food Consumers: What Do We Really Know about Them. British Food Journal, 114, 1157-1183.

Ditlevsen, K., Sandøe, P. & Lassen, J. (2019). Healthy food is nutritious, but organic food is healthy because it is pure: The negotiation of healthy food choices by Danish consumers of organic food. Food Quality and Preference, 71, 46-53.

Dončić, D., Popović, V. M., Lakić, Ž, Popović, D. B., Petković, Z. (2019). Economic analysis of wheat production and applied marketing management. Agriculture and Forestry, 65 (4): 91-100.

Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K. & Ortiz, R. (2016). Landrace germplasm for improving yield and abiotic stress adaptation. Trends in plant science, 21 (1): 31–42.

Frankin, S., Cna'ani, A., Bonfil, D. J., Tzin, V., Nashef, K., Degen, D., & Ben-David, R. (2023). New flavors from old wheats: exploring the aroma profiles and sensory attributes of local Mediterranean wheat landraces. Frontiers in Nutrition, 10, 1059078.

Fratianni, F., Cozzolino, A., d'Acierno, A., Nazzaro, F., Riccardi, R. & Spigno, P. (2020). Qualitative aspects of some traditional landraces of the tomato "Piennolo" (*Solanum lycopersicum* L.) of the Campania Region, Southern Italy. Antioxidants, 9(7), 565.

Guerrero, L., Guàrdia, M. D., Xicola, J., Verbeke, W., Vanhonacker, F., Zakowska-Biemans, S., & Hersleth, M. (2009). Consumer-driven definition of traditional food products and innovation in traditional foods. A qualitative cross-cultural study. Appetite, 52(2), 345-354.

Jevšnik, M., Hlebec, V. & Raspor, P. (2008). Consumers' awareness of food safety from shopping to eating. Food control, 19(8), 737-745.

Johansson, E., Prieto-Linde, M. L. & Larsson, H. (2021). Locally adapted and organically grown landrace and ancient spring cereals—A unique source of minerals in the human diet. Foods, 10(2), 393.

Jovovic, Z., Cizmovic, M., Lazovic, B., Maras, V., Bozovic, Đ., Popovic, T., Stesevic, D. & Velimirovic, A. (2011). The state of agricultural plant genetic resources in Montenegro. Agriculture and Forestry, 57(1), 33.

Lazaroiu, G., Andronie, M., Uţă, C. & Hurloiu, I. (2019). Trust management in organic agriculture: sustainable consumption behavior, environmentally conscious purchase intention, and healthy food choices. Frontiers in Public Health, 7, 340.

Le-Anh, T. & Nguyen-To, T. (2020). Consumer purchasing behaviour of organic food in an emerging market. International Journal of Consumer Studies, 44(6), 563-573.

Orsini, S., Costanzo, A., Solfanelli, F., Zanoli, R., Padel, S., Messmer, M. M., & Schaefer, F. (2020). Factors affecting the use of organic seed by organic farmers in Europe. Sustainability, 12(20), 8540.

Paull, J. (2015). GMOs and organic agriculture: Six lessons from Australia. Agriculture & Forestry, 61(1), 7-14.

Pieniak, Z., Verbeke, W., Vanhonacker, F., Guerrero, L., & Hersleth, M. (2009). Association between traditional food consumption and motives for food choice in six European countries. Appetite, 53(1), 101-108.

Puddephatt, J. A., Keenan, G. S., Fielden, A., Reaves, D. L., Halford, J. C., & Hardman, C. A. (2020). 'Eating to survive': A qualitative analysis of factors influencing food choice and eating behaviour in a food-insecure population. Appetite, 147, 104547.

Sills, S. J. & Chunyan, S. (2002). Innovations in Survey Research: An Application of Web–Based Surveys. Social Science Computer Review, 20 (1): 22–30.

Sinesio, F., Cammareri, M., Cottet, V., Fontanet, L., Jost, M., Moneta, E., & Grandillo, S. (2021). Sensory traits and consumer's perceived quality of traditional and modern fresh market tomato varieties: A study in three European countries. Foods, 10(11), 2521.

Sobal, J. & Bisogni, C. A. (2009). Constructing food choice decisions. Annals of behavioral medicine, 38(suppl_1), s37-s46.

Starr, G., Petersen, M. A., Jespersen, B. M. & Hansen, Å. S. (2015). Variation of volatile compounds among wheat varieties and landraces. Food chemistry, 174, 527-537.

Stolz, H., Stolze, M., Hamm, U., Janssen, M. & Ruto, E. (2011). Consumer attitudes towards organic versus conventional food with specific quality attributes. NJAS: Wageningen Journal of Life Sciences, 58(3-4), 67-72.

Velimirović, A., Jovović, Z., Perović, D., Lehnert, H., Mikić, S., Mandić, D., & Finetti-Sialer, M. M. (2023). SNP Diversity and Genetic Structure of "Rogosija", an Old Western Balkan Durum Wheat Collection. Plants, 12(5), 1157.

Villa, T. C. C., Maxted, N., Scholten, M. & Ford-Lloyd, B. (2005). Defining and identifying crop landraces. Plant genetic resources, 3(3), 373-384.

Wunderlich, S. & Gatto, K. A. (2015). Consumer Perception of Genetically Modified Organisms and Sources of Information. Advances in Nutrition, 6 (6): 842–851.

Zeven, A. C. (1998). Landraces: a review of definitions and classifications. Euphytica, 104 (2): 127–139.

Mir, Y.H., Shah, A.M., Shah, T.I., Bangroo, S.A., Jaufer, L., Kader, S., Mincato, R.L., Marković, R. (2023): Methodological challenges in estimating soil organic matter: A review. Agriculture and Forestry, 69 (4): 275-283. doi:10.17707/AgricultForest.69.4.19

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METHODOLOGICAL CHALLENGES IN ESTIMATING SOIL ORGANIC MATTER: A REVIEW

SUMMARY

Soil organic matter (SOM) plays a crucial role in soil health, fertility, and carbon cycling, making its accurate estimation essential for sustainable agriculture and ecosystem management. However, the quantification of SOM is fraught with methodological challenges that can introduce variability and uncertainty into assessments. Traditional techniques may lack specificity and accuracy, while advanced methods pose challenges related to calibration and standardization. The selection of an appropriate method is critical and requires careful consideration of soil characteristics, land use, and research objectives. This article reviews the key methodological challenges associated with estimating soil organic matter, aiming to provide an understanding of the complexities involved, and provides insights on the latest instrumentation for SOM measurements.

Keywords: sustainable agriculture; soil organic matter (SOM); ecosystem management; land use; instrumentation

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INTRODUCTION

The relevance of a landscape-scale approach to address environmental concerns in agricultural areas is being increasingly recognized by soil scientists with an emphasis on estimation of soil C stocks (Kader *et al.,* 2022; Sestras *et al.,* 2023). In terrestrial ecosystems, the largest stock of carbon is comprised by the soil surface, contributing significant proportion of organic carbon (OC) (Li *et al.*, 2022). Of this, soil organic matter (SOM) offers a prime source of organic carbon in soils, paving a way for the establishment and restoration of soil elements, carbon balance, and climatic conditions and environmental sustainability (Santoiemma, 2018).

Being the key parameter of agricultural soils, SOM is a practical index of soil fertility and soil quality and a prime factor in governing the dynamics of different agrochemicals in soil (Dhaliwal *et al*., 2019; Kader *et al.*, 2022). Furthermore, the physical, chemical and biological properties of different soil components are directly and indirectly affected by the SOM (Kader *et al.,* 2021; Kader *et al.,* 2023; Nannipieri and Eldor, 2009).

Comprehending the significance of organic matter, there is a call for regular monitoring of SOM for detection of changes in OC content, quality and its potential to sustain its purpose over time. Additionally, the SOM has proven an imperative parameter in directing soil fertilization as well as irrigation, thereby; a request for its determination by different fertility laboratories is apparent (Borase *et al.,* 2020; Kader *et al.*, 2022).

SOM encompasses a wide range of organic compounds, and no analytical method allows its accurate direct measurement. The determination of soil organic matter through conventional methodology relies on its oxidation, either by the utilization of strong oxidizing agents such as potassium dichromate in Tyurin or Walkley Black method with the use of conversion factor based on the assumption that SOM predominantly contains carbon (Shamrikova *et al*., 2022).

However, the principal limitation in estimating the total carbon by Walkley Black method is that it results in the partial recovery of organic carbon (Nie *et al*., 2021) and by dry combustion or EA method is the presence of inorganic carbon, most prominent problem in recently limed soils or calcareous soils. Therefore, accuracy of the analytical methods is critically important for the evaluation of short to medium term changes in soil organic carbon (SOC), such as, changes resulting from alternative agricultural management (Ramesh *et al.,* 2019).

Moreover, the assessment of soil organic matter on qualitative grounds in practical is still a challenging part. The involvement of humic acid to fulvic acid ratio and the absorbance ratio of alkaline soil extract at 400 and 600 nm wavelengths (E4/E6 or A400/A600) has been put forward for the assessment of SOM quality (Guillaume *et al.*, 2021), the fractionation being cantered on the principle of their differential solubility at distinct pH values (Kader and Jaufer, 2022).

However, majority of these determination methods are labour as well as time intensive. Therefore, there is a demand for employment of alternative approaches in determination of soil organic matter for detection of changes in soil organic matter both quantitatively as well as qualitatively and for provision of thorough and timely information regarding soil organic matter content at low expenditure along with an acceptable level of reliability.

Spectroscopy in detection of SOM

Recent developments in reflectance spectroscopy have led to its emergence as a method to improve the efficiency of soil carbon analysis and stock estimation (Nayak *et al.,* 2019). Visible near-infrared and mid infra-red reflection spectroscopic methods are cost effective, rapid, non- destructive and reproducible (Currà *et al.,* 2019). Mid infra-red spectroscopic approach has attained popularity due to its comparatively simple procedure of sample preparation along with the capability of evaluating dry soils directly (Guerrero *et al*., 2021). However, due to the appreciable sensitivity of the instrument regarding the homogeneity of the soil-matrix used, its applicability for determination of absolute concentrations is unsuitable. Usually, the outperformance of mid- infra-red spectroscopy to that of Vis- near infra-red has been concluded due to prevalence of more defined bands in case of mid-IR, thereby, performing better in quantification of soil organic matter as well as soil organic carbon (Mendes *et al.,* 2022).

An imperative requisite for the international databases have been marked through extensive reviews of all the accessible near-infra red techniques for soil quality assessment (Dharumarajan *et al*., 2023; S. Kader *et al.,* 2023; Youssef *et al.,* 2023). The suitability of Vis-infra red spectroscopic technique has been proven even for the independent set of validation as well as under outdoor circumstances. The reliable estimates for quantification of soil organic matter and soil organic carbon during laboratory analysis have been produced for Vis- Infra red spectroscopy (Aqua, Santos, and Chiang, 2019). However, because of abandoned variations in surface conditions of soil, the direct measurement of soil spectra in the field or employment of air-borne imaging spectrometry remains challenging.

The spectral mechanism of prediction varies with the soil sample population due to its reliance on decomposition phase of soil organic matter, nature of existing compounds and the impact of other allied factors viz., soil moisture, iron oxides and soil texture. The average R-squared value for the prediction of soil organic matter was observed to be 0.96 for mid- infra-red, 0.81 for near infra-red and 0.78 via visible spectroscopy (Shi *et al.,* 2023), reflecting a less accurate predictability of visible spectra.

The utilization of mid-IR to determine soil organic matter on qualitative basis dealing with the band assignments has been put forth by (Nasonova *et al*, 2022) after reviewing the applicability of mid infra-red spectroscopy for estimation of soil organic matter noted a general application of mid infra-red spectroscopy for both quantitative as well as qualitative analysis of soil. Spectrophotometric determinations of soil organic matter produced results with comparable precision and accuracy, improved performance, 60% fall in toxic reagent consumption and 91% decline in the generation of residue volume (Souza *et al.*, 2016). Spectral quality being the weightiest constraint in calibration of Vis-infra red and mid infra-red, require a pre-treatment of soil spectra quite ahead of spectroscopy model calibration for the reduction of interference contributed by variation in particle size distribution. Numerous examinations put forward revealed an enhanced accuracy of predictions made by Vis-near infra-red and mid infra-red spectroscopy after the application of various pre-treatment methods for thinning of particle size and noise effects.

Laser induced breakdown spectroscopy (LIBS)

Laser induced breakdown spectroscopy (LIBS), relying on the atomic emission, detects the characteristic spectral signature of carbon. A laser beam possessing a certain wavelength is focused on the sample that is to be analyzed, via a lens of 50 mm focal length forming the micro-plasma that emits a characteristic wavelength for each sample's elemental composition (Ebinger *et al.,* 2003). A high correlation of 0.96 for soils exhibiting similar morphology was observed while comparing the data from LIBS with that of data acquired from dry combustion (Senesi and Senesi, 2016; Cremers *et al.*, 2001). The initial study of Cremers *et al.* (2001) was further outstretched through the exploitation of Q-switched Nd-YAG laser at 266 nm wavelength having 23-mJ pulse along with the provision of ICCD for the measurement of carbon and nitrogen contents of soil samples from Oak Ridge and southwest Virginia mined lands possessing a range of 0.16%-4.3% of total carbon concentrations which were earlier acid washed and dried. Simple and multiple linear regression models were used for the development of calibration curves and data validation for laser induced breakdown spectroscopy (Cama-Moncunill *et al*., 2017). LIBS has proven an attractive and influential analytical tool unusual to conventional methodology due to some additive advantages such as no requisition of sample preparation, minimizing the disturbance of soil, it's comparative simplicity, less analytical time of the order of minutes, and the capability of analysing large number of samples every day associated with the cost efficiency. However, the challenges still exist that need to be addressed for its strengthened applicability such as the management of ablation and plasma formation, advancement and interaction with the adjoining environment, the attainment of equilibrium conditions in case of plasma, and excluding the effects of self-absorption that may interfere in portraying the signal intensity for elements at higher concentrations, thereby, making their quantification impractical.

Inelastic neutron scattering (INS) for SOM estimation

The novel inelastic neutron scattering (INS) technique for analysis of soil carbon relies on the gamma ray spectroscopy acquired from the interaction of fast neutrons with the nuclei of elements in soil (Yakubova *et al.*, 2017a). The inelastic neutron scattering technique analyzes the acquired spectral data for different peak intensities (counts), utilizing an established calibration curve, portrays the results instantaneously having units of kg C m^{-2}. An R-squared value of 0.99 has been observed while calibrating the INS system with synthetic soils containing a mixture of sand and a known quantity of carbon yielded. The significance of INS method to that of gold standard DC method has been confirmed where the speed of carbon content defining by the INS method was approximately 30-fold greater than the DC method (Yakubova *et al*., 2017b). The inelastic neutron scattering technique has a potential of analysing intact samples of soil samples on a large spatial scale, however, the cost incurred on instrumentation and transport are quite huge and the licenses from radiological control and properly skilled technicians are required.

Recent developments in SOM estimation

Assessment of different proficient analytical techniques such as Elemental analysis paired with Isotope ratio mass spectrometry (EA-IRMS), Pyrolysis - gas chromatography in combination with mass spectrometry (Py-GCMS), and Nuclear magnetic resonance spectroscopy (NMR) to determine soil organic matter has been performed in view of recent methodologies for soil organic matter studies (Santoiemma, 2018). EA-IRMS being the bulk assessment technique furnishes the data on average composition of elements detected and isotopic signature of a soil sample (Bianchi and Careri, 2021). The estimation of δ13C via EA-IRMS relates to the age of soil organic matter (Natali *et al*., 2018) accounting it to the relationship existing between fractionation of isotopes and microbial respiration, rapid mineralisation of 13C-poored constituents along with the decline in δ13C of CO_2 in the previous years as a consequence of fossil combustion. However, the accessibility of IRMS standards along with standardized methodology is the foremost goal to be addressed in present (Khatri et al., 2023). The coupling of analytical pyrolysis with gas chromatography-mass spectrometry (Py-GCMS) involves the decomposition of sample through pyrolysis/heating for the production of smaller compounds that can be separated by gas chromatography and detected by mass spectrometry (Bensidhom *et al.*, 2021).The identification of compounds present in the sample is done through a characteristic pattern of fragmentation, requiring huge database availability for recognition and elucidation of structure of chemical compounds. Py-GCMS improved the structural illustration of compounds, and with integration with other methods in multi-proxy approach, offered a high-resolution molecular data with regard to component analysis of organic matter. However, some ambiguity exists in pyrolyzed samples of original organic matter in relation to their representativeness.

NMR Spectroscopy for SOM estimation

NMR spectroscopic technique aids in the determination of atomic and molecular structure of the soil samples, the samples being de-mineralized accurately to evade the instrumental (Alexis *et al*., 2012). The employment of external magnetic fields possessing different strengths by different NMR spectrometer result in the distribution of the nuclei spins among different energy levels. Solid-state NMR spectroscopic method has proven an acceptable and influential analytical method for characterization of soil organic and for the determination of lability of soil organic matter fractions.13C NMR spectroscopic technique has been employed for the characterisation of humic acids after forest fires (Chen *et al*., 2020), for the characterisation of humic acids in bio-solid amended soils, for categorization of humic substances in per-humid montane forest soils, for characterisation of humic substances isolated from post-fire forest soils and for description of soil organic matter in virgin and Alnus forest. However, the reduced cross polarization efficiency for mobile constituents and non- protonated carbons along with the broad and overlapping of organic matter spectra pose a major challenge in future.

CONCLUSIONS

The recent advancements in estimating the soil organic matter promise a high precision along with the less time requirement for sample preparation and their subsequent analysis. The conventional laboratory estimation methods mark well accepted uncertainties and limitations, being laborious and time consuming and additionally complexed by the variability of organic carbon content along the profile both spatially and temporally. Therefore, the development of more rapid, accurate, robust, precise and cost-effective methodology with the requisition of no or minimal sample pre-treatment, minimizing the uncertainties and enhancing the organic carbon estimates and fluxes, is a crucial and dire need. The introduction of recent advancements is thus expected to empower a healthier quantification and validation of organic matter changes both temporally and spatially so as to address the issues of global climate change and terrestrial carbon management. Despite, of possessing myriads of advantages, a notable number of instrumental, methodological and procedural challenges might be faced that need to be figured out in present and future research.

REFERENCES

Alexis, M. A., Rasse, D. P., Knicker, H., Anquetil, C. & Rumpel, C. (2012). Evolution of soil organic matter after prescribed fire: A 20-year chronosequence. Geoderma, 189-190, 98-107.

Bensidhom, G., Arabiourrutia, M., Ben Hassen Trabelsi, A., Cortazar, M., Ceylan, S. & Olazar, M. (2021). Fast pyrolysis of date palm biomass using Py-GCMS. Journal of the Energy Institute, 99, 229-239.

Bianchi, F. & Careri, M. (2021). Mass spectrometry in food authenticity and traceability. In: GALANAKIS, C. M. (ed.) Food Authentication and Traceability. Academic Press.

Borase, D. N., Nath, C. P., Hazra, K. K., Senthilkumar, M., Singh, S. S., Praharaj, C. S., Singh, U. & Kumar, N. (2020). Long-term impact of diversified crop rotations and nutrient management practices on soil microbial functions and soil enzymes activity. Ecological Indicators, 114, 106322.

Cama-Moncunill, X., Markiewicz-Keszycka, M., Dixit, Y., Cama-Moncunill, R., Casado-Gavalda, M. P., Cullen, P. J. & Sullivan, C. (2017). Feasibility of laser-induced breakdown spectroscopy (LIBS) as an at-line validation tool for calcium determination in infant formula. Food Control, 78, 304-310.

Chen, H., Rhoades, C. C. & Chow, A. T. (2020). Characteristics of soil organic matter 14 years after a wildfire: A pyrolysis-gas-chromatography mass spectrometry (Py-GC-MS) study. Journal of Analytical and Applied Pyrolysis, 152, 104922.

Cremers, D. A., Ebinger, M. H., Breshears, D. D., Unkefer, P. J., Kammerdiener, S. A., Ferris, M. J., Catlett, K. M. & Brown, J. R. (2001). Measuring total soil carbon with laser-induced breakdown spectroscopy (LIBS). Journal of Environmental Quality, 30, 2202-2206.

Currà, A., Gasbarrone, R., Cardillo, A., Trompetto, C., Fattapposta, F., Pierelli, F., Missori, P., Bonifazi, G. & Serranti, S. (2019). Near-infrared spectroscopy as a tool for in vivo analysis of human muscles. Scientific Reports, 9, 8623.

Dhaliwal, S. S., Naresh, R. K., Mandal, A., Singh, R. & Dhaliwal, M. K. (2019). Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. Environmental and Sustainability Indicators, 1-2, 100007.

Dharumarajan, S., Gomez, C., Lalitha, M., Kalaiselvi, B., Vasundhara, R. & Hegde, R. (2023). Soil order knowledge as a driver in soil properties estimation from Vis-NIR spectral data – Case study from northern Karnataka (India). Geoderma Regional, 32, e00596.

Ebinger, M. H., Norfleet, M. L., Breshears, D. D., Cremers, D. A., Ferris, M. J., Unkefer, P. J., Lamb, M. S., Goddard, K. L. & Meyer, C. W. (2003). Extending the applicability of laser- induced breakdown spectroscopy for total soil carbon measurement. Soil Science Society of America Journal, 67, 1616-1619.

Guerrero, A., De Neve, S. & Mouazen, A. M. (2021). Chapter One - Current sensor technologies for in situ and on-line measurement of soil nitrogen for variable rate fertilization: A review. In: SPARKS, D. L. (ed.) Advances in Agronomy. Academic Press.

Guillaume, T., Bragazza, L., Levasseur, C., Libohova, Z. & Sinaj, S. (2021). Long-term soil organic carbon dynamics in temperate cropland-grassland systems. Agriculture, Ecosystems & Environment, 305, 107184.

Haque, F., Santos, R. M. & Chiang, Y. W. (2019). Using nondestructive techniques in mineral carbonation for understanding reaction fundamentals. Powder Technology, 357, 134-148.

Kader, S., Raimi, M. O., Spalevic, V., Iyingiala, A.-A., Bukola, R. W., Jaufer, L. & Butt, T. E. (2023). A concise study on essential parameters for the sustainability of scientific literature. Turkish Journal of Agriculture and Forestry, (2023) 47: 288-307. doi:10.55730/1300-011X.3087

Kader, S., Jaufer, L., Bashir, O. & Raimi, M. O. (2023a). A Comparative Study on the Stormwater Retention of Organic Waste Substrates Biochar, Sawdust, and Wood Bark Recovered from Psidium Guajava L. Species. Agriculture and Forestry, 69, 105-112.

Kader, S., Jaufer, L. (2022). A novel treatment for determining thermal conductivity of the soil substrates for selecting sustainable growing mediums in terms of thermal resistance. Agriculture and Forestry, 68, 111-118.

Kader, S., Chadalavada, S., Jaufer, L., Spalevic, V. & Dudic, B. (2022a). Green roof substrates-A Literature review. Frontiers in Built Environment, 8.

Kader, S., Jaufer, L., Shiromi, K. & Asmath, A. (2021). Comparison of physical and chemical properties to find the alternative substrate material for the betterment of green roof technology. 2021. The international Academic Forum (IAFOR).

Kader, S., Novicevic, R. & Jaufer, L. (2022b). Soil Management In Sustainable Agriculture: Analytical Approach For The Ammonia Removal From The Diary Manure. Agriculture & Forestry/Poljoprivreda i Sumarstvo, 68, 69-78.

Kader, S., Spalevic, V. & Dudic, B. (2022c). Feasibility study for estimating optimal substrate parameters for sustainable green roof in Sri Lanka. Environment, Development and Sustainability, 1-27.

Khatri, P. K., Paolini, M., Larcher, R., Ziller, L., Magdas, D. A., Marincas, O., Roncone, A. & Bontempo, L. (2023). Validation of gas chromatographic methods for lavender essential oil authentication based on volatile organic compounds and stable isotope ratios. Microchemical Journal, 186, 108343.

Li, H., Wu, Y., Liu, S., Zhao, W., Xiao, J., Winowiecki, L. A., Vågen, T.-G., Xu, J., Yin, X., Wang, F., Sivakumar, B., Cao, Y., Sun, P. & Zhang, G. (2022). The Grain-for-Green project offsets warming-induced soil organic carbon loss and increases soil carbon stock in Chinese Loess Plateau. Science of The Total Environment, 837, 155469.

Mendes, W. D. S., Demattê, J. A. M., Rosin, N. A., Terra, F. D. S., Poppiel, R. R., Urbina-Salazar, D. F., Boechat, C. L., Silva, E. B., Curi, N., Silva, S. H. G., José Dos Santos, U. & Souza Valladares, G. (2022). The Brazilian soil Mid-infrared Spectral Library: The Power of the Fundamental Range. Geoderma, 415, 115776.

Nannipieri, P. & Eldor, P. (2009). The chemical and functional characterization of soil N and its biotic components. Soil Biology and Biochemistry, 41, 2357-2369.

Nasonova, A., Levy, G. J., Rinot, O., Eshel, G. & Borisover, M. (2022). Organic matter in aqueous soil extracts: Prediction of compositional attributes from bulk soil mid-IR spectra using partial least square regressions. Geoderma, 411, 115678.

Natali, C., Bianchini, G., Vittori Antisari, L., Natale, M. & Tessari, U. (2018). Carbon and nitrogen pools in Padanian soils (Italy): Origin and dynamics of soil organic matter. Geochemistry, 78, 490-499.

Nayak, A. K., Rahman, M. M., Naidu, R., Dhal, B., Swain, C. K., Nayak, A. D., Tripathi, R., Shahid, M., Islam, M. R. & Pathak, H. (2019). Current and emerging methodologies for estimating carbon sequestration in agricultural soils: A review. Science of The Total Environment, 665, 890-912.

Nie, X., Zhao, T. & Su, Y. (2021). Fossil fuel carbon contamination impacts soil organic carbon estimation in cropland. Catena, 196, 104889.

Ramesh, T., Bolan, N. S., Kirkham, M. B., Wijesekara, H., Kanchikerimath, M., Srinivasa Rao, C., Sandeep, S., Rinklebe, J., Ok, Y. S., Choudhury, B. U., Wang, H., Tang, C., Wang, X., Song, Z. & Freeman Ii, O. W. (2019). Chapter One - Soil organic carbon dynamics: Impact of land use changes and management practices: A review. In: SPARKS, D. L. (ed.) Advances in Agronomy. Academic Press.

Santoiemma, G. (2018). Recent methodologies for studying the soil organic matter. Applied Soil Ecology, 123, 546-550.

Senesi, G. S. & Senesi, N. (2016). Laser-induced breakdown spectroscopy (LIBS) to measure quantitatively soil carbon with emphasis on soil organic carbon. A review. Analytica Chimica Acta, 938, 7-17.

Sestras, P., Mircea, S., Roșca, S., Bilașco, Ș., Sălăgean, T., Dragomir, L. O., Herbei, M. V., Bruma, S., Sabou, C., Marković, R. & Kader, S. (2023). GIS based soil erosion assessment using the USLE model for efficient land management: A case study in an area with diverse pedo-geomorphological and bioclimatic characteristics. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 51, 13263-13263.

Shamrikova, E. V., Kondratenok, B. M., Tumanova, E. A., Vanchikova, E. V., Lapteva, E. M., Zonova, T. V., Lu-Lyan-Min, E. I., Davydova, A. P., Libohova, Z. & Suvannang, N. (2022). Transferability between soil organic matter measurement methods for database harmonization. Geoderma, 412, 115547.

Shi, L., O'rourke, S., De Santana, F. B. & Daly, K. (2023). Prediction of soil bulk density in agricultural soils using mid-infrared spectroscopy. Geoderma, 434, 116487.

Souza, D. M. D., Morais, P. A. D. O., Matsushige, I. & Rosa, L. A. (2016). Development of alternative methods for determining soil organic matter. Revista Brasileira de Ciência do Solo, 40, e0150150.

Yakubova, G., Kavetskiy, A., Prior, S. A. & Torbert, H. A. (2017a). Applying Monte-Carlo simulations to optimize an inelastic neutron scattering system for soil carbon analysis. Applied Radiation and Isotopes, 128, 237-248.

Yakubova, G., Kavetskiy, A., Prior, S. A. & Torbert, H. A. (2017b). Measurements of soil carbon by neutron-gamma analysis in static and scanning modes. JoVE (Journal of Visualized Experiments), e56270.

Youssef, B., Bouskri, I., Brahim, B., Kader, S., Brahim, I., Abdelkrim, B. & Spalević, V. (2023). The contribution of the frequency ratio model and the prediction rate for the analysis of landslide risk in the Tizi N'tichka area on the national road (RN9) linking Marrakech and Ouarzazate. Catena, 232, 107464.

De Meo, I., Apostol, E.N., Kallio, M., Marongiu, S., Badea, N.O., Paletto, A. (2023): Scientific café as a tool to involve civil society in the forestry sector: A methodological approach. Agriculture and Forestry, 69 (4): 285-301. doi:10.17707/AgricultForest.69.4.20

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SCIENTIFIC CAFÉ AS A TOOL TO INVOLVE CIVIL SOCIETY IN THE FORESTRY SECTOR: A METHODOLOGICAL APPROACH

SUMMARY

In recent decades, the social demand for the involvement of civil society in decision-making processes on environmental issues has been growing worldwide. Among the techniques for involving civil society, the Scientific Cafés are new and flexible tools aimed at ensuring effective communication between scientists, practitioners, civil society, and decision-makers on scientific topics in an informal and inclusive way. From 2004 to today, the Scientific Cafés are increasingly spreading in several scientific fields including forestry. The aim of this article is to analyze the state-of-the-art of Scientific Cafés at an international level and to propose a novel procedure for organizing Scientific Cafés to be adopted in the forestry sector. The literature review show that the first publication on “science cafés” dates back to 2004, while 73 peer-reviewed articles have been published to date (on average just under four articles per year). These publications consider the Scientific Cafés in four senses: science education; science communication; public engagement; and cultural investigation of science. The present study describes the approach followed in a public engagement activity carried out in the framework of ForestValue2 (Horizon Europe project). The approach is developed in five

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steps: i) identification of the scientific topics; ii) selection of the audience (general public or a specific target group of stakeholders); iii) Scientific Café organization (in person or virtually); iv) definition of the participatory technique; and v) definition of the outcomes. Finally, the approach is based on a few key aspects that create a successful Scientific Café: informality and accessibility of the events as well as a friendly, inclusive, and non-competitive environment.

Keywords: Public participation; transdisciplinary research; citizens' engagement; Café support group

INTRODUCTION

In the post-modern society, non-material values – e.g., social, cultural, and spiritual values – have assumed increasing importance compared to material values – e.g., raw materials and other tangible products – related to nature (Bhagwat, 2009). This transformation in the scale of individual values is society's response to the environmental and socio-economic changes that have occurred over the last 50 years (Slimak and Dietz 2006). In this context of change, the intrinsic value of nature finds a prominent role in scientific debate (Ansink *et al.* 2008) as-well-as the involvement of stakeholders and local communities in the decision-making process of natural resources management (Grumbine, 1994). At the same time, there has been a growing social demand for transparency and public participation in policy choices.

Thanks to an increased awareness among citizens of their rights and among decision makers of the need for a greater inclusiveness of all social actors, public participation in natural resource management is beginning to spread widely in all advanced-economy countries (Paletto *et al.* 2015a).

At political level, the UN Conference on Environment and Development held in Rio de Janeiro (1992) evidences the importance to involve the civil society in the decision-making process related to the management of natural resources by following the principles of public participation (Kant and Lee 2004). The Aarhus Convention, signed in 1998, empowered the role of citizens and civil society organizations in environmental matters and stated that the environmental rights are fundamental to involve citizens in policy issues. In particular, the Convention "on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters" stresses the importance of giving the opportunity for citizens to access environmental information favouring bottom-up processes (Paletto *et al*. 2012).

In this framework, one of the main concerns in involving civil society and stakeholders in decision-making processes are the gaps among scientists, civil society, stakeholders and decision makers (Ádám *et al.* 2015). These gaps are due to many reasons. One is the complexity of scientific language, which contributes to increase the breaks between scientist, civil society, and decision-makers. In certain contexts, the orientation to the present of politicians, the scientists' fair of political abuses of politicians, the stakeholders thinking more concentrated on their needs than on requirements based on ethics or concepts make difficult the interactions and the participative approaches (Elzinga, 2008; Kiteme and Wiesmann 2008). To solve these problems, we need to come up with methodswhich enable participatory deliberation and transdisciplinary research, yielding practical science-based outcomes (Angelstam *et al.* 2013; Hirsch Hadorn *et al.* 2008).

In the forestry sector, the involvement of social society can take place in different ways and with various procedures depending on the objectives to be achieved through the participatory process (Paletto *et al.* 2022). Participation can be characterized by different degrees of inclusiveness, from the simple sharing of information until empowerment in which social actors are part of the group of decision makers (Jones *et al.* 2000; Tabbush, 2004). In this sense, public participation in forestry sector should be seen as an opportunity to promote the social sustainability of decisions and strategies (European Commission, 2003).

Once the level of participation is established, there are different methodologies that can be used in the participatory process, based on the utilization of different techniques (Lecomte *et al.* 2005). The selection of a particular method or technique cannot be decided a priori but is a context-based choice, deeply related to the objectives of the specific process. Furthermore, the same method can be applied at different level of participation, to support and promote the development of the decision process.

Within this framework can be placed "Scientific Cafés" or "Scientific Aperitifs" – also known as Cafés o Apéritifs Scientifique in French-speaking countries. These events refer to the public discussion of socially pertinent questions and needs which have scientific content in an informal setting and are instruments to ensure effective and well-structured communication between scientists, practitioners, civil society, and decision-makers (Nesseth *et al*. 2021). Scientific Café are events in which the involvement of civil society is more engaging respect to purely informative events. Like hackathons and scenario workshops, in these events the common visitors asked scientific questions and tried to answer them with the help of scientists (Krüger *et al.* 2020). One of the first examples of events aimed at bridging the gap between civil society, scientists and politicians have been the citizen consensus conferences organized in Denmark. In these conferences the process took a few weeks, starting with a question posed to few participants around an issue of present research (Jensen, 2005).

In particular, Scientific Cafés are live forum events that host conversations about current science topics (Bazilio *et al*. 2016; Dijkstra, 2017). It can be considered informal occasions for general public – or a specific target group (e.g., high school students, university students, stakeholders of a sector) – to meet scientist, like researchers and experts, and to discuss scientific topics as-well-as ongoing and future projects. These forums are usually external of the academic environments and are appreciated by horizontality and recognition of knowledge and practices, multiple representativeness, and facility of interaction between panelists and audience (França *et al.* 2016). As emphasized by Dijkstra (2017), scientific cafés can have an influence in breaking down barriers to citizen participation as citizens increased their beliefs that they are able to participate meaningfully in techno-scientific issues.

In international literature, the first publication on "science cafés" dates back to 2004 (Adams, 2004), while to date there are 73 peer-review publications according to the Scopus database (https://www.scopus.com). The scientific literature considers Scientific Cafés in four different senses (Dallas, 2006): (i) science education, (ii) science communication, (iii) public engagement, (iv) cultural investigation of science. In public participation processes developed in the framework of forest sector, Scientific Cafés are set up as tools to connect civil society and scientist in a participatory and interactive way.

At the light of these considerations, the main objective of the present article is to show a novel and standardized procedure of Scientific Café organization and development to be adopted in the forestry sector, and to offer considerations that can be used in future experiences.

The research was developed in the ambit of the Horizon Europe Project ForestValue2. ForestValue2 brings together owners and managers of national and regional Research, Development and Innovation (RDI) programs in eleven Member States of the European Union (EU) and in one Associated Country with the aim to contribute to the alignment of national research and innovation policies. One of the main objectives of ForestValue2 is the implementation of a joint call, resulting in the funding of transnational collaborative Research & Innovation (R&I) projects. Considering this objective and the importance to guarantee an Open Science approach in the whole project, Scientific Cafés have been promoted and implemented with an aim to collect opinions and information from civil society on key scientific topics, and to share the civil society participants with accurate and reliable information, possibly correcting misinformation on the discussed topics.

METHODOLOGY

Reasons for organizing a Scientific Café

Taking into consideration the classification proposed by Dallas (2006), the Scientific Cafés in the decision-making processes related to forestry sector can be considered at the same time as science communication and as civil society engagement. In this sense, the general objective of a Scientific Café is to raise community awareness about relevant scientific topics such as advances in research, scientific issues, laws, and political milestones.

In particular, the specific objectives of a Scientific Café can be summarized as follows:

- To inform, raise awareness and transfer knowledge from the scientific community to citizens on a current scientific topic;
- To gather information, expectations, needs of citizens on a current scientific topic;
- To define intervention strategies to solve a problem related to a current scientific topic.

In some cases, more than one objective may need to be achieved, for example inform and at the same time gather information, expectations and needs from citizens.

Another crucial aspect to be considered are the desired outcomes produced by the realization of a Scientific Café. In this sense, one the main outcomes are the possibility of collecting quantitative and/or qualitative data from citizens. The opinions, expectations, needs of citizens are crucial for achieving joint outcomes shared between citizens, scientists, and politicians. Another key outcome of a Scientific Café is correctly informing and correcting misinformation on key scientific issues, disseminated by social networks or other channels of information diffusion.

Characteristics of Scientific Café and role of the support group

The main characteristics of an effective and successful Scientific Café can be summarized as follows (Navid and Einsiedel 2012):

- Open to everyone, no scientific and/or technical knowledge is necessary. This means that the Café is an inclusive event with free access;
- Takes place in public gathering places such as coffee shops, bars, restaurants, bookstores, galleries, outdoors or in an online user-friendly platform. Because of this it is an accessible and informal event;
- A two-way communication process in which the recipient (civil society) and the sender (scientists) are constantly changing role, making the Scientific Café an interactive meeting;
- The civil society takes knowledge on the subject and enable new understandings about the production of knowledge beyond the academy, making the Scientific Café an impactful event.

Scientific Café involves a Café support group, which comprises: i) a person with overall responsibility for the organization of Scientific Café; ii) a facilitator; iii) one or two experts with the role of speakers; iv) a rapporteur. One or more of the abovementioned figures may coexist in the same person.

The person responsible of the event must be thoroughly familiar with the local situation and maintain constant contact with the local area. She/he must also consider how to adapt the event to the local context. It is up to her/him, in collaboration with the group, to carry out, at the end of the event, the evaluation on the effectiveness of the approach taken.

The facilitator has a key role to improve the participatory environment, addressing the more specialized scientific issues in a simple and understandable way. As emphasized by other authors (Elsasser, 2007; Balest *et al.* 2018), the facilitator does not necessarily have knowledge about the scientific issue discussed, but she/he must be able to involve all participants in the debate – regardless of skills and knowledge resources – in the same way. In particular, it is at her/his hands to stimulate those who are less active during the debate.

The facilitators, who design and conduct the Cafés sessions, stimulate a series of parallel conversations around carefully crafted key questions important for the group (Schiele *et al*. 2022). Facilitators encourage all participants – especially experts – to find and use a common language, avoiding scientific terms, that will not be clear to the rest of participants. At the same time, experienced facilitators will be capable to monitor that exactness of meanings be preserved (Fischer *et al.* 2008).

The expert/s should be identified among researchers and professors with high skills in the chosen scientific topic. The experts have the role of conveying key information to bring the debate to life. The choice of experts must focus on empathic persons with a practical approach to the scientific issue. To reduce the speaking time but eventually have different views, it is preferable to involve two experts, respecting the gender balance (one female and one male). The experts must follow these recommendations during the Scientific Café: i) avoid complex language and technical words; ii) adopt an empathetic and engaging attitude; iii) give at least one example related to the everyday life of citizens; iv) leave the possibility of questions for doubts and curiosities from participants.

The facilitator is supported by a rapporteur who will draw up the final report of the event. The rapporteur transcribes the answers and comments of the participants, possibly making use of the recording of the event. To make this, it is necessary a prior consent of the participants. Rapporteur prepares documentation and informs the participants about the structure and organizational aspects of the event before the start of the Scientific Café. In addition, she/he also takes the responsibility for all logistical and secretarial aspects. The rapporteur must be a person trusted by the facilitator, and during the Scientific Café intervenes in the discussion only at the request of the facilitator.

It is very important that group members make their choices by working closely together.

Main steps of Scientific Café

The organization of Scientific Cafés is structured in five steps as shown in Figure 1.

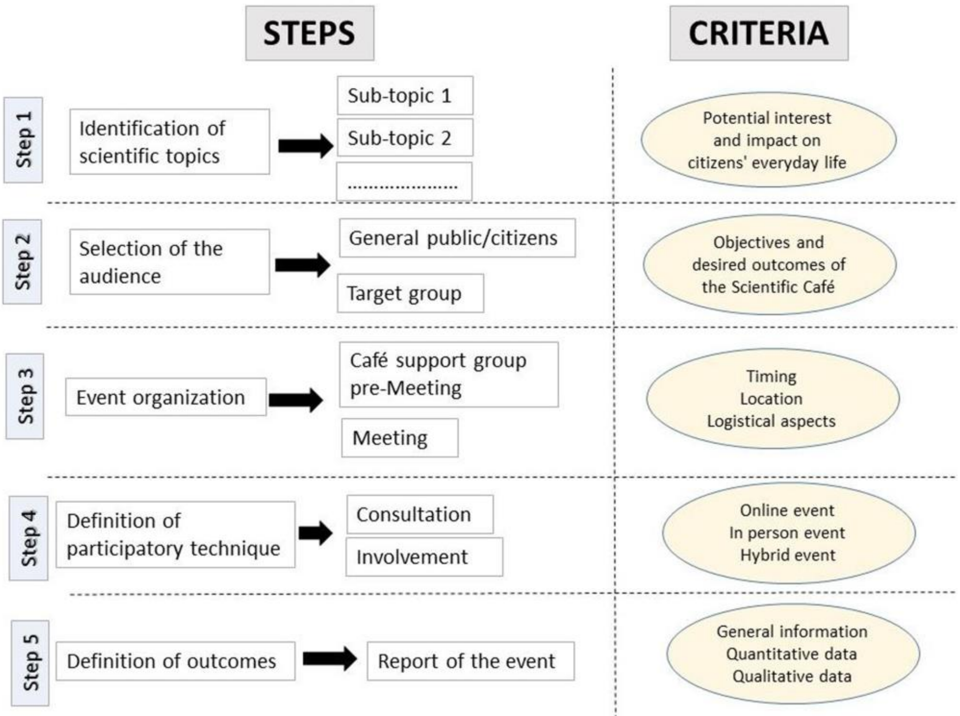

Figure 1. Description of the procedure for the organization of Scientific Cafés

Step 1 - Identification of the scientific topics and sub-topics

In the organization of a Scientific Café, the first step is the identification of the scientific topics to be discussed with the civil society during the event. The criteria used to identify the scientific topics are mainly three:

a) Relevance of the topic for a wide audience or for the selected target groups;

b) Information/disinformation on scientific topics through the mass media and social networks;

c) Concrete repercussions/impacts of the topic on citizens' well-being and lifestyles.

If the topic covers a wide scientific frame, it can be divided into key sub-topics of potential interest and impact on citizens' everyday life. The identification of the key sub-topics should be done in collaboration between responsible of the Scientific Café and experts of the chosen scientific topic.

Step 2 - Selection of the audience

The second step is the selection of the audience – e.g., general public or a specific target group – to address the Scientific Cafés.

Based on the main event's objectives and on the main desired outcomes to obtain from the Scientific Café, it is possible to identify the audience to be addressed to the event, choosing between the general public (citizens) or a specific target group (a segment of civil society or a group of stakeholders). In forestry sector, the citizens involved in the Scientific Café are mainly forest users. Therefore, they can be invited, for example, through forest user associations, trying to take into consideration all interests, such as wood production, products other than wood, rural development, outdoor recreation, quality of life/happiness, and biodiversity conservation. As highlighted in some studies on Scientific Cafés, the specific target group are mainly the following (Mayhew and Hall 2012; Bazilio *et al*. 2016; Balázs *et al*. 2020): middle and high school students; University and post-University students; stakeholders of a production sector; consumers of a particular product category.

When stakeholders are chosen as audience of the events, a stakeholder analysis process is necessary to identify all the different stakeholders belonging to the group (Reed *et al.* 2009; Paletto *et al.* 2015b). As an example, if the stakeholders of forest-based sector are the main audience for Scientific Cafés, the stakeholder identification will be an iterative process aimed at identifying all the categories of stakeholders. Starting from the institutional stakeholders and the main operators of the forestry sector, other representative parties are identified with the aim to take in consideration all the interests and opinions at stake (Figure 2). Based on this evaluation and choice, the publicity of Scientific Cafés will be direct.

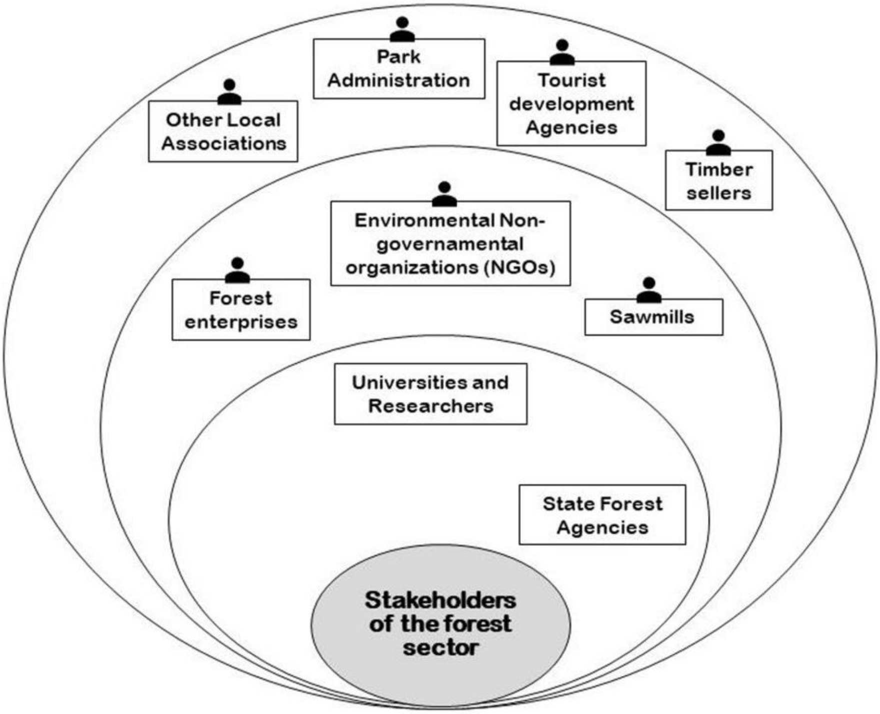

Figure 2. Stakeholders divided into target groups

Step 3 - Event organization

When realizing a Scientific Café there are some rules, based on the experience and on the objectives of the event, that mark the timing and manner of conducting the Café.

A fundamental part of the Café, necessary for the success of the event, is the organization of an online or in-person pre-meeting between the members of the Café support group. In this meeting, the event and the timing are organized in detail. The support group prepares a set of questions to address the most important aspects related to each scientific sub-topic (approximately 3-4 questions for each sub-topic). Then, the timing of the event is discussed: total length of Scientific Café; length of introduction; length of expert interventions; length of participatory process moderated by the facilitator. In addition, the support group defines the most important logistical aspects: i) the materials to be used, such as pc, projector, post-it, blackboards, pens/pencils, or other supporting materials; ii) the tools to be used, such as PowerPoint, videos, engagements tools as Slido (https://www.slido.com), Mentimeter (https://www.mentimeter.com/), Poll Everywhere (https://www.polleverywhere.com/), or others.

During the pre-meeting, the location of the event is discussed, evaluating firstly if the Scientific Café will be realized on an online event platform or in-person in a physical location.

Still in the pre-meeting, the dissemination channels to publicize the event are discussed. Furthermore the project for the Scientific Café poster or brochure and the choice of dissemination channels to invite the participants, such as website, social networks, personal invitations, mass media, or other channels, are done.

Regarding the duration of the event, Scientific Café format has an estimated duration of 90-100 minutes.

The optimal number of participants is less than 30 people; if there are more than 30 participants, it is preferable to divide the discussion into two sessions coordinated by two facilitators and with two rapporteurs. However, the final number of participants must be balanced between the different interest groups to avoid leading the discussion towards only one (or a few) objectives. To this end, all interest groups must be preliminarily invited but asking each institution/association to send only one representative. Besides, the event must be open to welcoming any additional and uninvited participants, always taking into consideration the balance between interests in the distribution of sessions.

At the opening of the event the facilitator introduces the Café concept, the topic, and the speaker(s) in a simple and sympathetic way to grab the attention of the participants. This moment takes around 5 minutes. Subsequently the speakers, who are the scientists, present the topic for approximately 10 minutes without any visual aids. After that, a drink or café is offered to the participants to create a relaxed and informal atmosphere. Then, the facilitator opens the floor for discussion, mainly in a question-and-answer format. This part of the Café takes around 45-50 minutes. During this part, the core of the Café, the facilitator stimulates the debate among the participants based on a pre-prepared set of questions, also with the use of some interactive tools useful to capture participants' views and to make everyone feel connected during the meeting.

<u>Step 4 - Definition of the participatory technique</u>

In order to define the participatory technique to be used, the first issue to be decided is whether to organize the Scientific Café: (1) online, on a web platform, or (2) in-person or (3) hybrid. After that, it is possible to modulate the most suitable participatory technique for the event.

The technique of involving the participants during the Scientific Café is structured in two phases. First there is the consultation of participants through a set of agree/disagree questions. After this, the involvement of the participants is realized through an open discussion and/or through the preparation of the problems and/or strategies tree.

In the consultation phase, the facilitator asks some simple close questions on the scientific sub-topics. In this phase, the use of some interactive tools could allow to capture participants' views. Specifically, the respondents answer to a set of pre-prepared questions through links.

In the phase of the participant involvement, which is crucial to the success of the event, two different techniques can be employed, whether the event is online or in-person.

In the online Scientific Café, the technique is the open discussion animated by the facilitator. This technique has the objective of involving all participants in expressing needs, expectations, and opinions regarding the scientific sub-topics. The open discussion is suitable to be applied also in outdoor in-person events.
For the in-person Scientific Café, one of the most suitable techniques to be used is the "Problem Tree" and "Strategy Tree" technique, which includes a few basic steps (Figure 3). Firstly, the facilitator and rapporteur distribute some post-it to the participants asking them to write down in 10 minutes what are – in their opinion – the main critical aspects related to the discussed sub-topics. The post-it notes are then given to the facilitator who distributes them on a blackboard by similarity of themes.

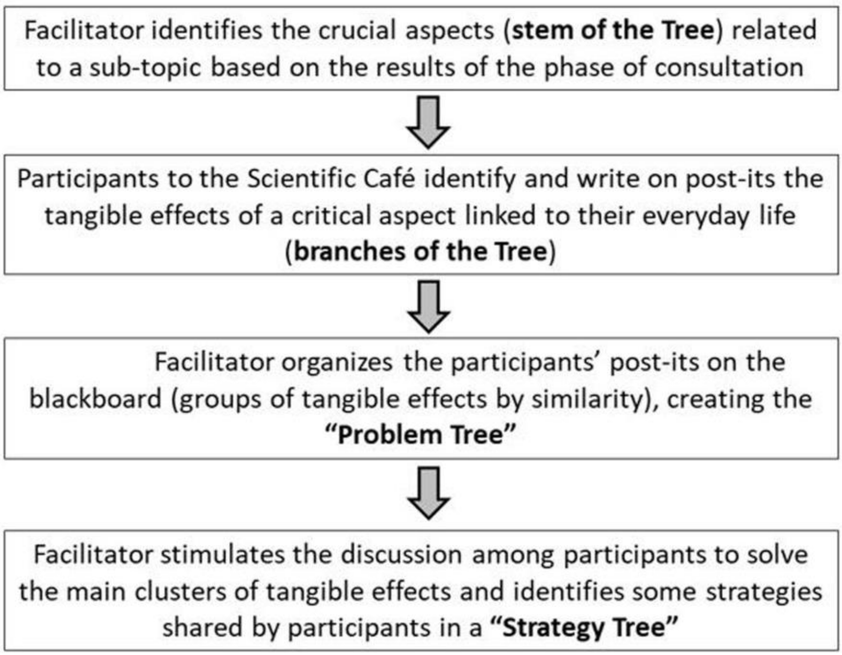

Figure 3. Steps of the participants' involvement during the Scientific Café.

The tangible effects of critical aspects in the everyday life identified by the participants are organized to create the "Problems Tree" (see Figure 4). The aim of the "Problems Tree" is to identify a core critical aspect (stem of tree) and its causes (roots) and tangible effects (branches) according to the logic, values, and consensus of citizens (Walubengo *et al.* 2019; Paletto *et al.* 2022). In the last part of this phase, the facilitator stimulates the discussion among participants to address/solve the main clusters of tangible effects, the branches of the "Problem Tree". Finally, the strategies identified by participants are organized in a "Strategies Tree" which is shared by all participants. For each "branch" of weakness, the participants define a strategy to overcome the weakness.

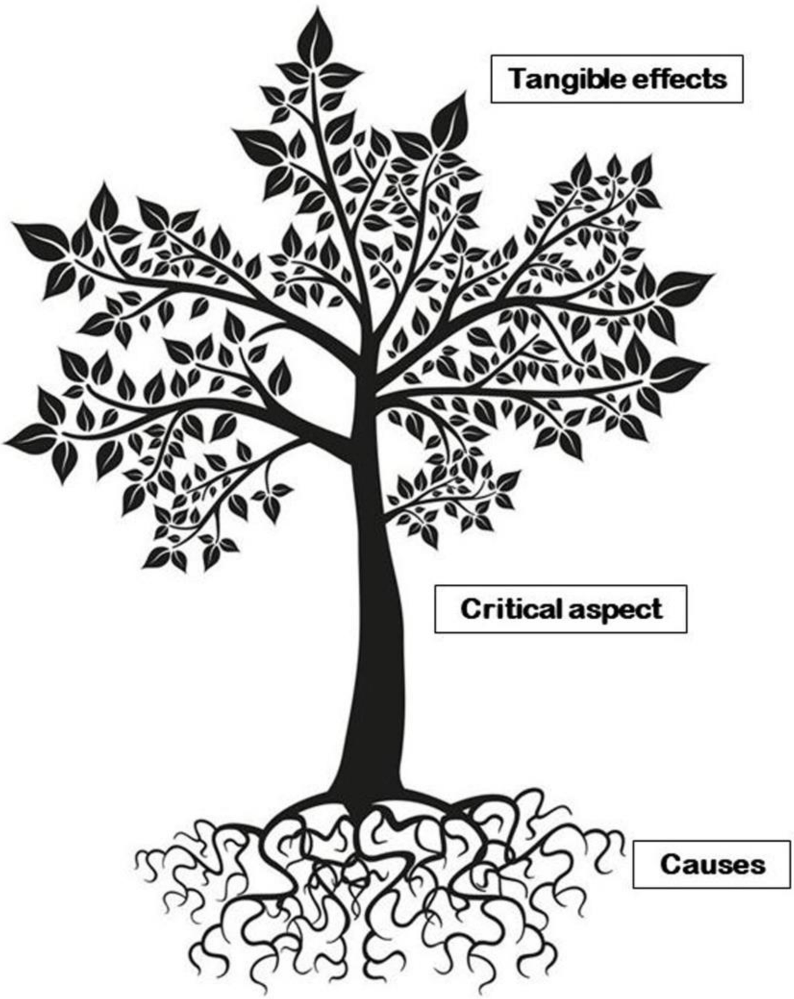

Figure 4. "Problems Tree" related to a scientific sub-topic

Step 5 - Definition of the outcomes

During Scientific Cafés both quantitative and qualitative information on the opinions, expectations, and needs of citizens related to key scientific topics and sub-topics are collected. Whatever the main objective of the event was, certain guidelines must be followed in the collection and dissemination of outcomes. In case multiple events are organized and different countries and partners are involved, it is essential that standardized reports are produced. The final document of a Scientific Café must contain the outcomes of the event. In general, this document is realized both in the language of the country and in English, to be shared and disseminated also out of the country's boundaries.

To this end, the information to be collected by the rapporteur for each event is the following: i) general information; ii) quantitative data; iii) qualitative data.

General information includes the duration and location of the event and number and typology of participants including those who supported the participatory process.

Quantitative data are the results collected using interactive tools such as EUSurvey, Slido, Mentimeter, Pool Everywhere or others.

Qualitative data are those derived from the "Problem tree" and the "Strategy tree" with highlighted tangible effects and the possible strategies to address/resolve clusters, indicated by participants in the event. In case of online events, results are collected using interactive tools to produce qualitative data.

DISCUSSION AND CONCLUSIONS

In the present article, we described the main objectives and characteristics of Scientific Cafés, and presented a novel procedure for the organization of these events in a simple and standardized way.

A research conducted by the Office of Science and Technology and Wellcome Trust (2001) has shown that non-specialists do not need to understand a great deal of the scientific details to be able to discuss social and ethical issues (OST–WT, 2001). In this sense, there is an increasing number of initiatives tending to promote the interaction between science and society, proposed from different fields. However, spaces in which this public debate could effectively take place are still missing. Scientific Cafés are the right spaces for these initiatives.

These events could support to recognize novel and critical issues for future research and policies in the forestry sector bringing together civil society, scientists, practitioners, and policy makers in the assessment of research fields. Furthermore, these events and the following outreach activities generate reflection and energy for pressing research needs.

In the forestry sector, there are issues like the role of forest for climate change mitigation and adaptation, the forest-based circular economy, where the knowledge production is raising, but the concept itself still lacks an evident analytical framework. Scientific Cafés are suitable tools to involve civil society in these issues, but in these cases, it is crucial that researchers seek to develop rooted case studies to inform and involve civil society. The different Scientific Cafes need to be adapted to local contexts and different situations, while following in principle the procedure developed.

It is important to verify that some main attributes are crucial for the success of the Scientific Cafés. When analysing examples of these events in the context of natural resources management it becomes clear that one of the most frequent causes of failure arises from not respecting these elements.

Navid and Einsiedel (2012) evidenced that the main reasons for the success of a Scientific Café can be attributed to a few factors. In their experience, the informality and accessibility of the events are essential to valuable transfer of knowledge and gather of information from civil society. This means firstly that timely information is available to all participants and the outcomes of the event are accessible to all, and secondly that people could talk freely, realizing that their opinions are often common opinions.

A friendly and non-competitive environment stimulates discussion and participants feel comfortable. Comfortability means creating an empathetic and engaging attitude, giving the voice to all participants, and stimulating those who are less active.

In five Science Cafés across Canada conducted to analyze civil society awareness of synthetic biology technology, Navid and Einsiedel (2012) demonstrated the effectivity of these tools to engage publics in dialogue about emerging technologies. Due to the Cafés interactive nature, they were able to acquire perspectives that may not have been captured through other approaches.

We would like to stress that interaction between scientists, experts and citizens must be promoted, and the debate among the participants must be stimulated. Reciprocal respect, willingness to listen and ability to compromise are important prerequisites for the success of Scientific Cafés. Another crucial attribute is the inclusiveness: Scientific Cafés must be open to everyone, and no scientific or technical knowledge is necessary to participate.

The communication strategies adopted during the Café need to be adapted to each target group, as different groups are sensitive to various arguments. The three "C" of a good communication and information of participants must be: i) Care; II) Clarity; and iii) Credibility: It means that the facilitator and also the rest of the Café support group need to care of participants through distribution of written materials, respect of time, and any kind of resources and facilities necessary to support participants. Clarity means using a language clear, simple, and accessible for all participants. Finally, credibility is ensured working with sound and reliable information regarding scientific topics.

Furthermore, several contextual factors, such as positive attitudes towards involvement and good relations amongst stakeholders, can enable successful implementation of Scientific Cafés. In this sense, fairness and inclusiveness need to be respected. All different views must be heard and respected, and attention must be given to the answers of each participant.

As highlighted by Balázs *et al.* (2020), Scientific Cafés are low-cost methods useful in the prioritization phase of a research agenda-setting exercise in a stimulating and convivial way. They used these tools to collect perceptions of research needs and to initiate social dialogue around green care in Hungary. They evidenced that maintaining interactions with civil society rests a crucial challenge when defining priorities of a research agenda. Within this context, Scientific Cafés with diverse participants can be useful to achieve a civil society role in co-producing research agendas. However, their experience in using Scientific Cafés for research agenda setting shows that mainly citizens of a specific target group (e.g., students; institutional stakeholders; professionals) tend to become engaged, rather than general public.

We would like to mention in this study that while there are attributes that can support the success of a scientific café, there are also negative circumstances that can cause its failure.

A study by Mizumachi *et al. (*2011) investigates the approaches in engaging with the civil society in a Scientific Café of 19 early career scientists in Japan. The research evidence that anxiety about dialogue with the citizens may be the greatest barrier for scientists in a Scientific Café. The Japanese experience failed for the reluctance among scientists to interact with the large public. In particular, scientists thought that organizing and holding a Scientific Café was too demanding in terms of time and resources and did not perceive the benefit of the event.

Another important element that must be taken in consideration when organizing a Scientific Café is that not everyone would like to participate. Furthermore, inequities in who is involved and how, and who is not involved, inevitably create biased knowledge production and unequal power relations (Balázs *et al.* 2020). The choice of the right tools and the right way of conducting Café is crucial for the success of the event, demonstrating the high responsibility of scientist who organize Cafés.

Tycova *et al.* (2023) developed Scientific Cafés to reach citizens living and working in the upper Malše river catchment (Czech Republic) that hosts the only remaining naturally reproducing freshwater pearl mussel population in the country. They evidence that among communication strategies for managing citizens and target groups in the forestry sector events such as Scientific Cafés highlighting the current issues and showing better practices are extremely helpful.

Among recommendations of these authors, there is the need to build mutual trust with the target groups. Building a relationship with participants includes providing an opportunity to discuss their needs and expectations and to express points of view. Furthermore, these authors evidence that the feeling of being controlled too much demotivates participants to get involved in the Scientific Cafés. Besides, they highlight that their experience showed that if participants are properly and in advance informed about the events, they are more likely to ask for consultation and are more willing to cooperate.

Finally, we would like to emphasize the importance of having different case studies and experiences available to refine and test the validity of the procedure described. In this sense, the future steps of the study will be to test the proposed methodology in different forestry contexts.

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REFERENCES

Ádám, J.I., Cerovac, I., Gajinov, T., Kalina, V., Paris, L. & Zwitter, Ž. (2015). Science cafés for a sustainable future. Transdisciplinary communication to understand the impacts of climate change on cultural heritage in the Danube River Basin. In: Brumat S. (ed.), DIAnet International School Proceedings 2015. The role of cultural heritage for the sustainable development of the Danube Region, Gorizia 14th-23rd March 2015, Trieste: EUT Edizioni Università di Trieste, pp.86-105.

Adams J.U. (2004). How to start a science café. Scientist 18(10): 50-51.

Angelstam, P., Andersson, K., Annerstedt, M., Axelsson, R., Elbakidze, M., Garrido, P., Grahn, P., Jönsson, K.I., Pedersen, S., Schlyter, P., Skärbäck, E., Smith, M. & Stjernquist, I. (2013). Solving problems in social–ecological systems: Definition, practice and barriers of transdisciplinary research. Ambio 42: 254-265.

Ansink, E. Hein, L. & Hasund, K.P. (2008). To value functions or serices? An analysis of ecosystem valuation approaches. Environmental Values 17: 489-503.

Balázs, B., Horváth, J. & Pataki, G. (2020). Science-society dialogue from the start: Participatory research agenda-setting by Science Cafés. European Journal of Futures Research 8: 5.

Balest, J., Hrib, M., Dobšinská, Z. & Paletto A. (2018). The formulation of the National Forest Programme in the Czech Republic: A qualitative survey. Forest Policy and Economics 89: 16-21.

Bazilio, A., Ryan, A. & Welborn, J. (2016). Science Cafés. An affordable, easy-to-implement model that introduces young girls to STEM-related topics, careers, and role models. Science Scope 40(3): 14-17.

Bhagwat, S.A. (2009). Ecosystem services and sacred natural sites: Reconciling material and non-material values in nature conservation. Environmental Values 18(4): 417-427.

Dallas, D. (2006). Café Scientifique—Déjà Vu. Cell 126: 227-229.

Dijkstra, A.M. (2017). Analysing Dutch Science Cafés to better understand the science-society relationship. Journal of Science Communication 16(1): 1-17.

Elsasser, P. (2007). Do “stakeholders” represent citizen interests? An empirical inquiry into assessments of policy aims in the National Forest Programme for Germany. Forest Policy and Economics 9: 1018–1030.

Elzinga, A. (2008). Participation. In: Hadorn, G.H., Hoffmann-Riem, H., Biber-Klemm, S., Grossenbacher-Mansuy, W., Joye, D., Pohl, C., & Zemp, E. (Eds.). Handbook of transdisciplinary research. Dordrecht: Springer, pp.345-359.

European Commission (2003). Natura 2000 and forests “Challenges and opportunities” — interpretation guide Office for Official Publications of the European Communities. available online at http://ec.europa.eu/environment/nature/info/pubs/docs/nat2000/n2kforest_en.pdf

Fischer, S., Smith, G.T. & Cyders M.A. (2008). Another look at impulsivity: A meta-analytic review comparing specific dispositions to rash action in their relationship to bulimic symptoms. Clinical psychology review 28(8): 1413-1425.

França, C.G., Carvalho, D.L.A.F., & Da Silva Alves, R.A. (2016). Science cafe and maker culture: an emergent technology theme discussed in a transversal way. In: EDULEARN16 Proceedings, IATED, pp.1531-1538.

Grumbine, R.E. (1994). What is ecosystem management? Conservation Biology 8(1): 27-38.

Hirsch Hadorn G. (2008). The Emergence of Transdisciplinary as a Form of Research. In: Hadorn, G.H., Hoffmann-Riem, H., Biber-Klemm, S., Grossenbacher-Mansuy, W., Joye, D., Pohl, C., & Zemp, E. (Eds.). Handbook of transdisciplinary research. Dordrecht: Springer, pp.19-39.

Jensen, C.B. (2005). Citizen Projects and Consensus-Building at the Danish Board of Technology: On Experiments in Democracy. Acta Sociologica 48(3): 221-235.

Jones, P., Burgess, J. & Bhattachar, D. (2000). An evaluation of approaches for promoting relevant authority and stakeholder participation in European Marine Sites in the UK. Report, Environment and Society Research Unit.

Kant, S. & Lee, S. (2004). A social choice approach to sustainable forest management: an analysis of multiple forest values in Northwestern Ontario. Forest Policy and Economics 6(3-4): 215-227.

Kiteme, B.P., & Wiesmann, U. (2008). Sustainable river basin management in Kenya: Balancing needs and requirements. In: Hadorn, G.H., Hoffmann-Riem, H., Biber-Klemm, S., Grossenbacher-Mansuy, W., Joye, D., Pohl, C., & Zemp, E. (Eds.). Handbook of transdisciplinary research. Dordrecht: Springer, pp.63-78.

Krüger, K., Molas, A., Jiménez, L., Hjelt, J., Pekkola, E., Stenvall, J., & Kobza, N. (2020). RRI in three countries: Finland, Poland and Spain-Catalonia. In: Krüger, K., Molas, A., Jiménez, L., Hjelt, J., Pekkola, E., Stenvall, J, Stasik, A., Dańkowska, A., & Kobza, N. (Eds.), Responsible Research & Innovation in three countries: Finland, Poland and Spain-Catalonia. dia-e-logos Discussion Paper 02/2020. Oldenburg/ Barcelona.

Lecomte, N., Martineau-Delisle, C. & Nadeau, S. (2005). Participatory requirements in forest management planning in Eastern Canada: A temporal and interprovincial perspective. The Forestry Chronicle 3: 398–402.

Mayhew, M.A. & Hall, M.K. (2012). Science Communication in a Café Scientifique for High School Teens. Science Communication 34(4): 546–554.

Mizumachi, E., Matsuda, K., Kano, K., Kawakami, M. & Kato, K. (2011). Scientists' attitudes toward a dialogue with the public: a study using "science cafes". Journal of Science Communication 10: A02.

Navid, E.L & Einsiedel, E.F. (2012). Synthetic biology in the Science Café: what have we learned about public engagement? Journal of Science Communication 11(4): 1-9.

Nesseth, N.M., Henson, A.M. & Barriault, C.L. (2021). A Framework for Understanding the Nature of Questions Asked by Audience Participants at Science Cafés. Frontiers in Education 6: 674878.

OST–WT (Office of Science and Technology and Wellcome Trust) (2001). Science and the public: a review of science communication and public attitudes towards science in Britain. Public Understanding of Science 10: 315–330

Paletto, A., Becagli, C., Geri, F., Sacchelli, S. & De Meo, I. (2022). Use of participatory processes in wood residue management from a circular bioeconomy perspective: an approach adopted in Italy. Energies 15: 1011.

Paletto, A., Cantiani, M.G. & De Meo, I. (2015a). Public Participation in Forest Landscape Management Planning (FLMP) in Italy. Journal of Sustainable Forestry 34(5): 465-482.

Paletto, A., Ferretti, F. & De Meo, I. (2012). The role of social networks in forest landscape planning. Forest Policy and Economics 15: 132-139.

Paletto, A., Hamunen, K. & De Meo, I. (2015b). Social network analysis to support stakeholder analysis in participatory forest planning. Society & Natural Resources 28: 1108-1125.

Reed, M.S., Graves A., Dandy N., Posthumus H., Hubacek K., Morris J., Prell C., Quinn C.H. & Stringer L.C. (2009). Who's in and why? A typology of stakeholder analysis methods for natural resource management. Journal of Environmental Management 90: 1933-1949.

Schiele, H., Krummaker, S., Hoffmann, P. & Kowalski, R. (2022). The "research world café" as method of scientific enquiry: Combining rigor with relevance and speed. Journal of Business Research 140: 28.

Slimak, M.W. & Dietz, T. (2006). Personal values, beliefs, and ecological risk perception. Risk Analysis 26: 1689-1705.

Tabbush, P. (2004). Public money for public good? Public participation in forest planning. Forestry 2: 145-156.

Tycova, I., Ourednik, R., & Spisar, O. (2023). Communication with local community and stakeholders as a key activity to protect oligotrophic streams with freshwater pearl mussel. Limnologica 98: 126048.

Walubengo, W.W., Kyalo D.N. & Mulwa A.S. (2019). Analytical review of application of Problem Tree Analysis as a project design tool for enhancing performance of community based in Kenya. European Journal of Business and Management Research 4(6): 1-7.

INSTRUCTIONS TO AUTHORS

The "Agriculture and Forestry" journal publishes original scientific papers, review papers, short communications on agriculture, veterinary medicine, forestry, biology and other natural sciences. It is the endeavour of the journal to give place to papers of high scientific quality and international interest, authored by all the scientists from the South Eastern European Region, as well as other international scientist in order to stimulate contacts and exchange of knowledge fostering scientific productivity.

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Full research papers should include the following sections:

- Author/s name/s, Title with **DOI number**

The author's name should be placed above the title and with the author's appellation and affiliation in a footnote at the bottom of the front page. Author(s) affiliation should indicate name and address of institution, including the e-mail address of the corresponding author. Title should provide a concise but also an informative synthesis of the study (recommended not more than 100 characters including spaces). Short title (not more than 70 characters) should also be provided in order to be included in the header of the Manuscript. Ensure that the title contains the most important words that relate to the topic.

- Abstract

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions. Include the keywords and phrases you repeated in your abstract.

- Key words

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

Main text of the manuscript includes the following sections:

- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

- RESULTS followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. More details you can find in the Annex to the INSTRUCTIONS TO AUTHORS / Bibliographic style on the web page of the Journal: www.agricultforest.ac.me.

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