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INNOVATIONS IN SUSTAINABLE AGRICULTURE – BRIDGING SCIENCE AND PRACTICE

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SECTION 1

PLANT PRODUCTION AND PROCESSING

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THE ROLE OF GRAFTING IN PLANT GROWTH OF TWO KAPYIA-TYPE CULTIVARS

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ABSTRACT

This study investigated the effects of grafting on plant growth development in two kapyia pepper cultivars, Ariadni F1 and Kaptur F1. A three-year experiment was conducted using three rootstocks: 'SM Tant' (*Capsicum annuum* x *C. chinense*), 'Vital Paprika' (*Capsicum annuum* x *C. chinense*), and '6210' (a cross of two pepper genotypes). All treatments were arranged in a randomized block design with four replications, under greenhouse and open-field conditions. Plant growth was evaluated every 10 days, beginning 15 days after transplanting and continuing until the onset of full harvest. Measurements included stem diameter below the first bifurcation. Data were analyzed using two-way ANOVA with repeated measures to assess the effects of grafting and the environment. Results showed that grafting combinations exhibited different growth rates after transplanting. The rootstock 'SM Tant' demonstrated the fastest and most consistent development from transplanting to ripening, supporting a favourable balance between vegetative and productive biomass.

Key words: *grafting, kapyia peppers, plant growth, stem diameter.*

INTRODUCTION

Vegetable grafting is a promising technique for successful production under biotic and abiotic stress conditions. Grafting in pepper is less prevalent globally, compared to other vegetables, due to its compatibility only with other *Capsicum* species (Gaion et al., 2018). Further complexity is added by the specific interactions between rootstock, scion, and the environment.

Several studies have shown that grafting can enhance vegetative growth in pepper plants to varying degrees, particularly in terms of plant height (Colla et al., 2008; García-Rodríguez et al., 2010; López-Marín et al., 2012; Rizani et al., 2021; Shu et al., 2016). These benefits are often attributed to the rootstock's robust root system, which provides water and nutrients more effectively than the scion's roots, as well as the delivery of endogenous hormones to the shoots. This combination boosts the vigor of the scion and, ultimately, its productivity. However, a review of the literature indicates inconsistent results regarding the effects of different rootstocks on the height of grafted plants. Some studies demonstrated reduced plant growth in grafted plants except in some cases when the same plants were grown under abiotic or biotic stress conditions (García-Rodríguez et al., 2010; López-Marín et al., 2017; Soltan et al., 2017), while other experiments discovered reduced plant growth when some particular rootstock was used (Allagui et al., 2013; Leal-Fernández et al., 2013). Regarding the stem diameter, as for the plant height, similar findings were obtained in these studies.

Therefore, the aim of this study was to investigate the effects of grafting on plant growth development in two kapyia pepper cultivars Ariadni F1 and Kaptur F1, grafted on three different rootstocks.

MATERIAL AND METHODS

A three-year experiment was conducted using two kapyia pepper cultivars Ariadni F1 and Kaptur F1 grafted on three rootstocks: 'SM Tant' (*Capsicum annuum* x *C. chinense*), 'Vital Paprika' (*Capsicum annuum* x *C. chinense*), and '6210' (a cross of two pepper genotypes). Nongrafted plants were used as control. All treatments were arranged in a randomized block design with four replications, under greenhouse (GH) and open-field (OF) conditions. Plant growth dynamics was evaluated every 10 days, beginning 15 days after transplanting and continuing until the onset of full harvest. The measurements were performed on 5 plants from each replication, starting from the soil to the apex, with measuring tape. The rate of growth was expressed as a percentage of deference subtracted from the final plant height that was recorded at the end of the harvest at the beginning of October. The stem diameter was measured below the first bifurcation with a digital calliper.

Data was analysed using two-way ANOVA with repeated measures to assess the effects of grafting and the environment. Tukey's Honest Significant Difference post-hoc test was used to test the difference between stages. After verifying the significance of the interaction for each variable, a one-way ANOVA was performed. Means were compared by the Fisher's least significance difference (LSD) test and Duncan's multiple range test at $P < 0.05$. All statistical analyses are performed in SPSS version 23.0.0.0 (SPSS, 2015).

RESULTS AND DISCUSSION

The grafting combinations developed at different rates after transplanting. The statistical analyses demonstrated that both Grafting status and Environmental factors had a significant influence on the plant growth, as evidenced by the within-subjects results ($F (4,92) = 145.16$, $p < .001$). Moreover, the between-subjects effects revealed highly significant interactions among the evaluated grafted combinations for the evaluated growth stages ($F (4,48) = 216.342$, $p < .001$), as presented in Table 1.

Table 1. Growth dynamics of plant height in bell pepper type graftings (cm)

Grafting status		Envir.	10 DAT	20 DAT	30 DAT	40 DAT	50 DAT
ARIANDI F1	0	OF	18.95±0.13**	25.28±0.10**	33.45±0.13**	37.15±0.13**	37.98±0.15**
		GH	19,28±0.30**	29,63±0.59**	40,90±0.34**	46,13±0.28**	50,10±0.34**
	1	OF	22.58±0.10**	31.35±0.45**	35.65±2.37**	42.48±0.34**	41.08±0.36**
		GH	24.68±0.36**	36.10±0.37**	46.83±0.34**	49.30±0.25**	54.30±0.22**
	2	OF	19.63±0.13**	26.38±0.10**	36.43±0.10**	40.48±0.13**	35.23±0.40**
		GH	20.98±0.17**	31.13±0.33**	39.10±0.34**	42.93±0.34**	54.98±0.47**
	3	OF	21.08±4.85**	31.23±0.17**	31.08±0.28**	35.05±0.13**	35.43±0.15**
		GH	24.75±0.39**	32.95±0.47**	40.28±0.41**	45.55±0.21**	56.63±0.33**
KAPTUR F1	0	OF	26.23±0.21**	38.48±0.57**	40.73±0.25**	44.70±0.18**	49.20±0.43**
		GH	27.45±0.44**	44.38±0.85**	53.80±0.43**	57.58±0.31**	64.83±0.47**
	1	OF	24.60±0.08**	31.85±0.13**	41.48±0.13**	45.78±0.10**	53.28±0.41**
		GH	24.63±4.89**	36.45±0.42**	51.73±1.26**	56.40±0.64**	67.38±0.68**
	2	OF	26.25±0.13**	36.45±0.29**	38.10±0.26**	42.75±0.13**	44.88±0.26**
		GH	29.58±0.36**	40.45±0.66**	49.90±0.89**	56.68±0.26**	66.80±0.37**
	3	OF	28.85±0.13**	35.60±0.08**	38.25±0.21**	42.45±0.17**	44.10±0.25**
		GH	30.78±0.21**	36.78±0.62**	46.65±0.39**	51.53±0.75**	59.05±0.58**

Data are means ± standard error (n = 4). Symbols indicate statistically significant differences within subjects; * $p < 0.05$; ** $p < 0.001$; Repeated Measures ANOVA; Tukey's Honest Significant Difference post-hoc test

For Ariadni F1 the observations are illustrated in Figure 1. It can be noticed that, in nearly all growth stages, the non-grafted hybrids exhibited a slower growth dynamic compared to all grafted combinations, except during certain stages when grafted onto the rootstock '6210'

(R3), which displayed an exceptionally vigorous growth rate during the first ten days after transplanting. Since both of the investigated hybrids belong to the *kapia*-type peppers, prolonged fruit setting is expected. This characteristic may explain the growth stagnation observed in Ariadni F1 during the last two growth stages (9.7% and 2.7% at 40 and 50 DAT, respectively). However, contrary to this expectation, Kaptur F1 exhibited the most rapid growth during the second ten-day period (Figure 2), which was also the only stage in which these plants were taller than all their grafted combinations. In both cases—that is, in the variants of both hybrid cultivars—the rootstock SM Tant induced the fastest plant growth across almost all stages, while maintaining the most balanced development from transplanting to full maturity. This balance ensured a favourable relationship between vegetative and reproductive growth.

No statistically significant differences were observed between the grafted and non-grafted variants for any of the hybrids grown under open-field conditions. In contrast, under greenhouse conditions, the plants of 'Ariadni' F1/'6210' were significantly taller than those of the non-grafted 'Ariadni' F1, whereas the plants of 'Kaptur' F1/'6210' were significantly shorter than both the non-grafted 'Kaptur' F1 and its other grafted variants. These findings indicate that the effect of grafting on plant height is largely dependent on the specific scion–rootstock interaction and the growing environment, suggesting that greenhouse conditions may enhance or suppress graft vigor depending on the compatibility between the hybrid and the selected rootstock.

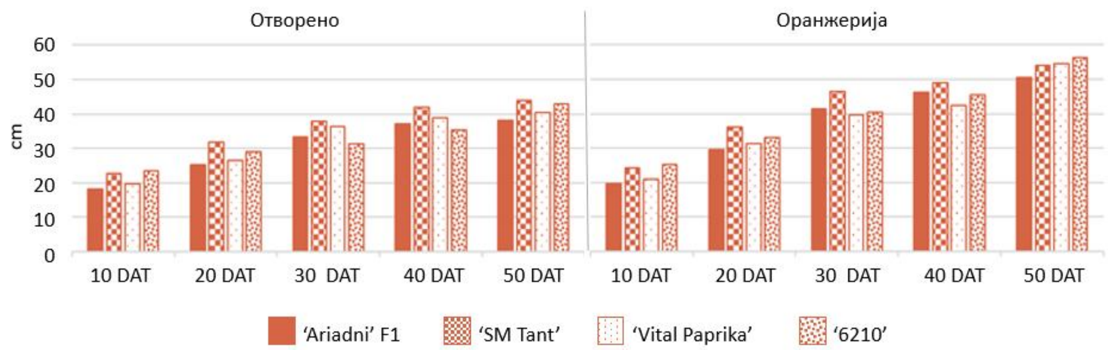

Figure 1. Actual (cm) and growth dynamics of Ariadni F1 non-grafted and grafted on different rootstocks (R) measured in five-time intervals after transplanting (DAT)

The observations from our study are consistent with the findings of Doñas-Uclés et al. (2015), who observed faster initial growth in non-grafted plants, while only the combination Palermo/Jalapeno showed significantly taller plants at the end of the season. Similar results were reported by Aidoo et al. (2019), indicating that grafting responses depend on production conditions, environmental stress, and rootstock scion compatibility. These results confirm that the effect of grafting on growth dynamics is highly variable and strongly influenced by both the growing environment and the scion–rootstock interaction.

The two-way ANOVA with repeated measures, for stem diameter, revealed that there were statistically significant differences within and between subjects regarding Grafting status and Environment (Table 2). In both hybrids, nongrafted variants had higher values compared to their grafted combinations.

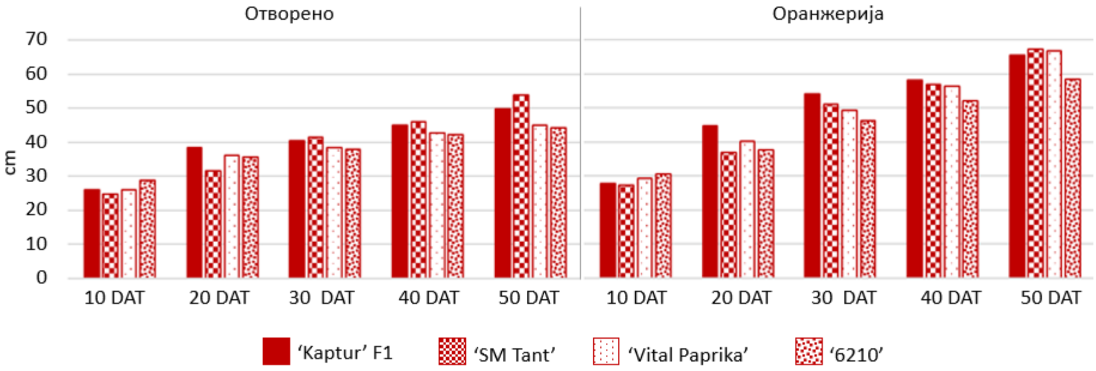

Figure 2. Actual (cm) and growth dynamics of Kaptur F1 non-grafted and grafted on different rootstocks (R) measured in five-time intervals after transplanting (DAT)

Table 2. Growth dynamics of stem diameter in the investigated kapiya type grafted peppers

Grafting status		Envir.	10 DAT	20 DAT	30 DAT	40 DAT	50 DAT
ARIANDI F1	0		6.10 ± 0.01 **	7.75 ± 0.01 **	8.03 ± 0.03 **	8.77 ± 0.02 **	10.75 ± 0.45 **
		GH	6.37 ± 0.11 **	8.32 ± 0.01 **	9.43 ± 0.01 **	13.32 ± 0.03 **	20.98 ± 1.04 **
	1	OF	4.26 ± 0.01 **	6.86 ± 0.05 **	7.47 ± 0.01 **	8.18 ± 0.04 **	11.39 ± 0.12 **
		GH	5.38 ± 0.13 **	7.32 ± 0.01 **	8.87 ± 0.04 **	11.15 ± 0.09 **	12.51 ± 0.03 **
	2	OF	4.78 ± 0.02 **	6.65 ± 0.09 **	6.86 ± 0.03 **	7.45 ± 0.01 **	10.17 ± 0.15 **
		GH	5.29 ± 0.03 **	7.36 ± 0.01 **	9.26 ± 0.01 **	12.10 ± 0.02 **	13.12 ± 0.03 **
	3	OF	4.81 ± 0.03 **	6.23 ± 0.04 **	6.47 ± 0.02 **	8.02 ± 0.02 **	9.06 ± 0.47 **
		GH	5.42 ± 0.06 **	7.51 ± 0.02 **	8.22 ± 0.04 **	12.01 ± 0.09 **	13.84 ± 0.06 **
KAPTUR F1	0	OF	6.85 ± 1.49 **	7.56 ± 0.02 **	9.09 ± 0.03 **	9.86 ± 0.02 **	13.96 ± 0.08 **
		GH	6.47 ± 0.15 **	8.15 ± 0.05 **	9.84 ± 0.06 **	10.07 ± 0.07 **	12.26 ± 0.05 **
	1	OF	4.61 ± 0.04 **	6.24 ± 0.04 **	8.09 ± 0.03 **	8.74 ± 0.16 **	10.43 ± 0.19 **
		GH	5.47 ± 0.15 **	7.29 ± 0.05 **	9.46 ± 0.23 **	11.41 ± 0.23 **	11.49 ± 0.05 **
	2	OF	4.70 ± 0.02 **	5.64 ± 0.03 **	6.23 ± 0.02 **	6.77 ± 0.02 **	9.61 ± 0.06 **
		GH	5.21 ± 0.04 **	6.57 ± 0.03 **	7.79 ± 0.08 **	11.14 ± 0.10 **	12.82 ± 0.05 **
	3	OF	4.64 ± 0.16 **	6.83 ± 0.01 **	6.82 ± 0.04 **	7.47 ± 0.02 **	11.72 ± 0.13 **
		GH	5.14 ± 0.04 **	7.25 ± 0.04 **	8.99 ± 0.03 **	10.27 ± 0.03 **	12.72 ± 0.06 **

Data are means ± standard error (n = 4). Symbols indicate statistically significant differences within subjects; * $p < 0.05$; ** $p < 0.001$; Repeated Measures ANOVA; Tukey's Honest Significant Difference post-hoc test

Research assessing stem diameter in grafted pepper plants is relatively scarce. In most available studies, grafted combinations have shown a tendency toward reduced stem thickness under standard cultivation conditions (Ergun & Aktas, 2018; Shu et al., 2016) or when grown in *P. capsici*-infested soils (García-Rodríguez et al., 2010). Nevertheless, some investigations reported comparable stem diameters between grafted and non-grafted plants (Allagui et al., 2013; García-Rodríguez et al., 2010). The effect of the rootstock itself also appears inconsistent—certain rootstocks have been associated with an increase (Leal-Fernández et al., 2013), while others with a decrease (Orosco-Alcalá et al., 2021) in stem diameter—whereas in several cases, no significant influence of the rootstock on this parameter was detected.

CONCLUSION

Based on our results, it can be concluded that grafting had consistent positive effect on the final plant growth of the investigated scion – rootstock combinations. The variation in different growth stages was not solely influenced by the grafting status and the growing environment. Instead, the onset of fruit setting played a critical role, disrupting the balance between vegetative and productive growth. The rootstock 'SM Tant' provided optimal growth balance in both of the grafted cultivars.

Overall, the impact of grafting on plant height and stem diameter in pepper plants is complex and depends on various factors, including the specific rootstock - scion combination, as well as the environmental conditions.

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INVESTIGATION ON PEPPER SEED QUALITY PRODUCED UNDER DIFFERENT FERTILIZER AND TRICHODEMA TREATMENTS

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ABSTRACT

This study aimed to assess the seed quality characteristics of *Capsicum annuum* L. produced under three different fertilizer schemes and Trichoderma harzianum (TRICH) treatments. The experiment included three pepper varieties: Amfora, Sivria, and Vezen Lut. Key quality parameters, including moisture content, analytical purity, germination rate and absolute seed mass were evaluated. Seed analyses were performed at the Laboratory for Seed Testing at the Institute of Agriculture in Skopje. Pre-sowing and post-harvest evaluations were carried out to determine the qualitative status of the seeds and the effects of fertilization and Trichoderma treatment. The seed material was divided into two groups: untreated (TRICH/0) and treated with Trichoderma (TRICH/1). Separate analyses were conducted for each fertilization and treatment combination. The results revealed that fertilization schemes significantly influenced seed quality metrics across all cultivars. In both regions, the germination rates for Amfora and Sivria varieties without TRICH treatment (TRICH/0) ranged from 96.3% to 99.0%. However, in the Kochani region, varieties treated with Trichoderma (TRICH/1) exhibited slightly higher germination rates, ranging from 97.8% to 99.2%. For untreated seeds (TRICH/0) the 1,000-seed mass ranged from 6.72 to 7.25 g, while for treated seeds (TRICH/1) it ranged from 6.36 to 7.13 g. The Vezen Lut variety showed relatively lower germination rates (90% - 97.3%), likely due to its classification as a local landrace commonly cultivated by farmers.

Key words: *pepper seed, quality, germination*

INTRODUCTION

Seed quality plays a crucial role in enhancing agricultural yield, serving as a foundational requirement for successful crop production (Poštić et al., 2019; Rahman, 2014). High-quality seed is typically characterized by high viability, purity, and germination capacity; when combined with proper agronomic practices, it maximizes productivity and profitability (Letting, 2018). In pepper production, a major challenge lies in improving seed vigor and germination, as well as development of healthy seedlings with well-structured root systems.

Biological agents such as *Trichoderma* spp. have attracted increasing interest due to their role in promoting plant growth and resilience. By colonizing roots, they enhance nutrient uptake, strengthen defense mechanisms, and improve tolerance to abiotic and biotic stresses, with some strains reported to increase yields by up to 30% (Howell, 2003; Benítez et al., 2004). In parallel, fertilization strategies—mineral, organic, or mixed—also influence seed development, potentially improving quality traits important for crop establishment and productivity.

Seed quality testing provides an objective assessment of parameters, such as germination energy, total germination, purity, and seed mass, ensuring the production of vigorous planting material (Tančić-Živanov et al., 2020). The present study aims to investigate key seed quality indicators specifically germination energy, total germination, moisture content, analytical purity and absolute seed mass, across three different pepper varieties, produced under various fertilizer schemes and Trichoderma treatments.

MATERIAL AND METHODS

The research focused on the seed quality parameters of three pepper varieties: Amfora, Sivria, and Vezen Lut, produced in two different locations: Skopje and Kocani. Seed multiplication trials were organized under five treatments including three fertilization schemes (MIN – Mineral fertilizer, ORG – Organic fertilizer and MIX – Combination of mineral and organic fertilizers), untreated (TRICH/0) and treated (TRICH/1) plots with *Trichoderma harzianum* (trade name Trianum-P, Koppert). The evaluation of the seed quality indicators was performed at the Laboratory for Seed Testing at the Institute of Agriculture in Skopje, in accordance with the MKC EN ISO/IEC 17025 standard, that regulates the testing of quality properties of seeds in agricultural plants. Before the establishment of the multiplication trials on the field, the quality properties of the input seed material were analysed with standard laboratory tests. At the end of the season, after the postharvest operations, the entire multiplied seed material was submitted to laboratory testing. The quality analyses of the obtained seed material were performed by regions and separately for two harvest layers (I- first and II- second), for maximum quality(Alan & Eser, 2007). The assessment was based on parameters such as germination (both energy and total germination), moisture content, seed purity, and absolute seed mass. To assess germination energy and total germination, 100 seeds from each variety were selected. The germination test followed a standard laboratory method, utilizing filter paper moistened with distilled water, with four sets of 100 seeds each. The seeds were placed in Petri dishes and maintained at a temperature of 25-30°C.The samples were incubated for periods of 7 (GE) and 14 days (TG). On the seventh day of incubation, germination energy (GE) was assessed, while on the fourteenth day, total germination (TG) was evaluated, specifically counting the number of typical seedlings.

RESULTS AND DISCUSSION

The initial quality analysis of the input material revealed that the overall quality of the seeds was relatively high for Amfora and Sivrija. Vezen Lut exhibited low germination (40.2%), consistent with its status as a local landrace typically maintained by farmers for dehydration (Drvoshanova et al., 2023). Therefore, the complete seed material from this variety was treated with Trichoderma in order to ensure successful multiplication. The quality of seeds obtained after the multiplication trials is presented below.

Table 1. Seed quality parameters of the input seed material for investigated pepper varieties

Variety	Seed purity (%)	Germination rate (%)	Weight of 1000 seeds (g)	Moisture (%)
AMFORA	98.7	98.0	7.13	12.0
SIVRIA	99.5	97.5	7.16	12.2
VEZEN LUT	91.1	40.2	7.98	13.1

Across multiplication trials, fertilization regimes significantly influenced seed quality (Tables 2–3). In line with Constantin et al. (2025), who reported positive fertilization effects on pepper seed yield, our results demonstrated that mixed (MIX) fertilizer consistently produced the highest germination rates across varieties. Seeds obtained from plants treated only with mineral fertilizes had the lowest performance for all tested quality parameters.

In all tested varieties, the germination rate was highest in seeds obtained from plants treated with mixed fertilizers (organic and mineral). In this study the effect of the Trichoderma treatment can only be indirectly considered, since it promotes abiotic and biotic stress resilience to the plants, contributing to higher fruit and seed yield.

Table 2. Seed quality parameters of the multiplied seed material of pepper varieties - not treated with *Trichoderma harzianum* in Skopje and Kochani

Variety	Treatment	Germination (%)	1000-seed weight (g)	Moisture (%)
AMFORA	MIN - I (Skopje)	94.3	5.68	9.1
	MIN - I (Kochani)	94.7	5.81	11.9
	ORG – I (Skopje)	92.0	6.38	9.9
	ORG – I (Kochani)	95.8	6.57	11.8
	MIX – I (Skopje)	99.0	6.72	11.9
	MIX – I (Kochani)	96.3	7.33	10.7
	MIN - II (Skopje)	91.0	5.56	10.5
	MIN - II(Kochani)	91.0	5.82	11.3
	ORG – II (Skopje)	98.3	5.98	11.8
	ORG – II (Kochani)	94.8	6.88	12.8
	MIX – II (Skopje)	98,5	6.66	10.9
	MIX – II (Kochani)	96.3	6.91	11.6
SIVRIA	MIN - I (Skopje)	92,5	5.88	10.10
	MIN - I (Kochani)	94,5	7.25	10.95
	ORG – I (Skopje)	96,7	6.97	12.2
	ORG – I (Kochani)	91,5	6.07	12.1
	MIX – I (Skopje)	99	6.08	10.5
	MIX – I (Kochani)	98,8	6.41	10.75
	MIN - II (Skopje)	92,2	6.55	11.40
	MIN - II(Kochani)	91.5	7.16	11.05
	ORG – II (Skopje)	97	6.62	11,4
	ORG – II (Kochani)	91,5	5.92	11.05
	MIX – II (Skopje)	98,8	6.08	11.05
	MIX – II (Kochani)	95,5	6.23	11.77

Notable differences in germination rates were observed depending on whether the plants were treated with *Trichoderma* or not, with variations evident among the tested pepper varieties. Specifically, for the untreated varieties (TRICH/0), germination values ranged from 96.3% to 99.0% for the Amfora and Sivria varieties in both regions (Table 2).

Data presented in table (3) showed higher germination rates, ranging from 97.8% to 99.2%, but only in the trial from Kocani region. Additionally, the mass of 1000 seeds ranged from 6.72g to 7.25g for untreated varieties (TRICH/0) and from 6.36g to 7.13g for those treated with Trichoderma (TRICH/1). The variety vezen lut demonstrated a relatively lower germination rate between 90% and 97.3%.

Table 3. Seed quality parameters of the multiplied seed material of pepper varieties (treated with *Trichoderma harzianum*) in Skopje and Kochani

Variety	Treatment	Germination (%)	1000-seed weight (g)	Moisture %
AMFORA	MIN - I (Skopje)	94,0	5.67	11.6
	MIN - I (Kochani)	91,8	6.57	11.2
	ORG – I (Skopje)	90,0	5.78	12.3
	ORG – I (Kochani)	95,8	6.57	11.8
	MIX – I (Skopje)	96,3	5.92	12.7
	MIX – I (Kochani)	97,8	6.01	10.9
	MIN - II (Skopje)	94,3	5.47	10.8
	MIN – II (Kochani)	93,7	6.08	10.70
	ORG – II (Skopje)	94,5	5.98	11.8
	ORG – II (Kochani)	95,7	5.62	11.25
	MIX – II (Skopje)	97,5	6.50	11.8
	MIX – II (Kochani)	96	6.10	10.45
SIVRIA	MIN - I (Skopje)	96	5.88	11.4
	MIN - I (Kochani)	97	6.13	12.7
	ORG – I (Skopje)	95	6.27	11.4
	ORG – I (Kochani)	97.5	6.58	12.55
	MIX – I (Skopje)	97.5	6.05	11.55
	MIX – I (Kochani)	99.2	7.13	10.9
	MIN - II (Skopje)	97	5.72	12.3
	MIN - II(Kochani)	96.3	6.91	11.85
	ORG – II (Skopje)	96.5	6.22	12.4
	ORG – II (Kochani)	95.5	6.52	11.7
	MIX – II (Skopje)	98.7	6.3	12.15
	MIX – II (Kochani)	96.3	7.01	11.6
VEZEN LUT	V – I (Skopje)	97.3	7.46	11.25
	V – I (Kochani)	94	8.3	12.15
	V – II (Skopje)	96.7	7.18	12.05
	V – II (Kochani)	90	7.9	11.45

CONCLUSIONS

It is evident that the various fertilizing schemes gave different results on the quality parameters. In all tested varieties, the germination rate is highest in seeds obtained from plants treated with mixed fertilizers (organic and mineral). The effect of the *Trichoderma* treatment can only be indirectly considered, since it promotes abiotic and biotic stress resilience to the plants, contributing to higher fruit and seed yield. on germination rates suggests its potential role in improving seed quality by promoting healthier seedling development. Comprehensive seed testing is essential for evaluating parameters such as germination percentage, physical purity, and moisture content, ensuring that high-quality seeds are provided to farmers.

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TECHNOLOGICAL SOLUTIONS FOR EXTENDING THE CROPPING PERIOD IN GREENHOUSES OF THE SOUTHEAST REGION

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ABSTRACT

This review, based on analysis of national statistics, relevant documents, and international best practices, identifies key issues in greenhouse production in the Southeast region. First, overlapping production cycles in glasshouses and PE tunnels create seasonal surpluses—up to 200 tons during peak seasons (May-June, September-November)—causing market saturation, reduced prices, and increased waste. Second, basic agricultural practices limit yield potential and shorten cropping seasons, indicating the need for modern techniques. Third, a lack of traceability and good agricultural practices (such as crop rotation) reduces yield and product quality, underscoring the importance of documented agricultural protocols. To address these challenges, we recommend implementing modern microclimate control systems that improve automation and resource efficiency, thus reducing operational costs. Advanced heating, cooling, and ventilation management decreases energy use and provides stable growing conditions, even in extreme weather, allowing for an extended cropping period. This would enable crop production beyond traditional seasons, boosting market potential and ensuring a consistent supply of fresh produce. In the Southeast region, where PE tunnel and outdoor production overlap, adopting these technologies would optimize timing between greenhouse and outdoor production. Additionally, returning cash crops like tomatoes, cucumbers, zucchini, and hot peppers to protected environments could enhance productivity and market quality, stabilizing the supply and economic returns for local producers.

Key words: *PE tunnels, tomato, pepper, cucumber.*

INTRODUCTION

The natural climatic advantages of the modified Mediterranean climate in certain regions of our State include high radiation levels in autumn and winter, with a predominance of direct solar radiation over diffuse radiation during the numerous clear days, mild winter air temperatures, and overall climatic stability. By implementing appropriate microclimate control measures, simple greenhouses, such as PE tunnels—which represent the most common type of greenhouse—can achieve a similar cost-to-benefit balance as industrial-type greenhouses Castilla & Montero, 2008). Perhaps the primary advantage of advanced greenhouse technology lies in ensuring production continuity and stability throughout most of the year. However, production in greenhouses became unprofitable during the years. Similar to the countries in the region that shared the same history of closed markets (Draghici et al., 2021), the massive glasshouses complexes ranging from 6 to 36 hectares per location (330 ha in total) built by the end of 80s are now obsolete. A significant portion of the costs for vegetables harvested successively, such as tomatoes, peppers, or cucumbers, is attributed to seed materials and labor. Hybrid seed materials, designed for extended multi-month or even multi-year harvests, become economically unviable when used for shorter harvesting periods. For (Shukla et al., 2024)instance, with a three-month harvesting season and average yields ranging between 6.5–10 kilograms per square meter, the profitability of seeds engineered to yield 60 kilograms or more per square meter diminishes significantly. To enhance income per unit area, it is essential to adopt structures (Abid et al., 2024) and technologies that extend the harvest season (Bazgaou et al., 2023) and/or focus on crops with shorter growing cycles, allowing for multiple cycles in a year (Popsimonova et al., 2022). Expanding the production of flowering, cold-resistant crops is another viable option. There are applicable and relatively inexpensive technological alternatives to the current production methods that align with the principles of sustainable agriculture, such as smart and/or resilient agriculture. These approaches are established as strategies to address the challenges posed by climate change, which will be explored further in this paper.

METHODOLOGY

To achieve the objectives of this study, a comprehensive methodological framework was employed, integrating multiple approaches to gather and analyze data. The following methods were applied:

Desk Review of Statistical Data, Literature, and Documents

A thorough review of statistical data, reports, and documents related to fresh produce production, trade, and distribution in North Macedonia and the Southeast region.

Examination of data from national and regional sources to understand the trends, challenges, and opportunities within the fresh produce sector.

Sector-Specific Data Collection

Detailed desk research and analysis of information provided by the Department for Vegetables at the Field Crops Sector, Ministry of Agriculture, Forestry and Water Economy (MAFWE).

Incorporation of data from the Agency for Financial Support of Agriculture and Rural Development to evaluate the financial mechanisms and support schemes available to producers.

Economic Context Analysis

Desk review of statistical data and literature focusing on the broader economic context of North Macedonia. This mainly includes evaluating trade dynamics relevant to the fresh produce sector.

Extensive Literature Review

In-depth analysis of existing academic and industry literature to identify trends and innovations in fresh produce production globally.

Examination of international best practices and successful case studies in greenhouse and protected cultivation, tailored to the specific economic, climatic, and agricultural contexts of North Macedonia.

Comparative Analysis of International Practices

Identification and assessment of global success stories in fresh produce production, distribution, and trade.

Analysis of their applicability and relevance to North Macedonia's agricultural landscape, with a focus on adaptability to local climatic and economic conditions.

Integrated Approach to Data Interpretation

Combining insights from diverse sources to create a holistic understanding of the fresh produce sector in North Macedonia and the Southeast Region.

Synthesizing statistical data, institutional reports, and international practices to propose actionable recommendations for improving production, trade, and sustainability.

By leveraging these methodological approaches, this study ensures a robust and well-rounded analysis, providing insights that are both evidence-based and context-specific. The integration of local data with international best practices aims to deliver practical recommendations for enhancing fresh produce production and distribution in North Macedonia.

RESULTS AND DISCUSSION

Conditions for Fresh Vegetable Production in Protected Areas

Greenhouses are stationary facilities designed for off-season cultivation under controlled conditions, including active heating, cooling, dehumidification, and carbon dioxide enrichment. These facilities have solid foundations and are not based on soil cultivation. Depending on the covering material, greenhouses are classified into different types (Baille, 2001). According to EU definitions of agricultural activity and agricultural land, as well as priorities for environmental and resource protection (including soil), areas used for greenhouse production under the Common Agricultural Policy (CAP) are not eligible for direct payments (Shukla et al., 2024). In contrast, tunnels (walk-in tunnels) are structures that passively utilize solar energy with minimal microclimatic control. These facilities are mobile, and production occurs directly on the ground.

The term "early vegetable production" is now outdated, originating nearly 50 years ago when polyethylene films enabled the widespread cultivation of off-season vegetables in PE tunnels. During the same era, greenhouses were constructed within state-owned agricultural enterprises in the southern regions of the former SFRY. Thanks to favorable climatic conditions, vegetables grown in these areas reached markets earlier than those cultivated outdoors. This early production initiative positioned Macedonia as a leading horticultural producer in the Balkans, with capacities reaching approximately 10,000 hectares of tunnels and over 300 hectares of greenhouses at their peak. Individual farmers producing early-season vegetables achieved high market prices, establishing vegetable gardening as a highly profitable agricultural activity.

Over time, global advancements in greenhouse technology have made year-round off-season vegetable production possible (Wielgat et al., 2024; Nauta et al., 2023). This development renders the term "early vegetable production" obsolete. To ensure a continuous supply of fresh vegetables to urban centers, production methods have shifted toward maximizing yields per square meter. Soil cultivation was largely abandoned three decades ago (Fuentes-Peñailillo et al., 2024), with vegetables now grown on substrates or hydroponically. Modern greenhouse facilities incorporate fully automated systems for managing microclimatic conditions, including regulated nutrition, active ventilation, cooling, shading, additional lighting, dehumidification, and carbon dioxide enrichment. Efforts are also underway to utilize sustainable energy sources for heating and cooling. In contrast, the only modernization in local protected production has been the introduction of micro-irrigation systems.

The most profitable "cash crops" for greenhouse production include tomatoes, lettuce, peppers, cucumbers, and young spinach. However, in our protected areas, less profitable crops such as potatoes, cabbage, and industrial peppers are often grown. Cabbage, which has become the leading crop, defies both production and market logic, though it may be justifiable for cultivation in unheated or lightly heated tunnels. For cash crops like tomatoes, yields remain low due to inadequate technology and short harvesting periods. Greenhouse tomato yields reach just 10 kg m^{-2}, comparable to unheated greenhouse yields in Almería, Spain (Castilla & Montero, 2008), but five to six times lower than greenhouse yields in the Netherlands.

Climatic conditions once considered advantageous for "early vegetable production" now act as a limiting factor for year-round cultivation. Seasonal extremes, exacerbated by climate change, necessitate heating during winter and active ventilation or cooling during summer. These requirements demand specialized equipment and energy inputs, which render production unprofitable in facilities with short harvesting seasons.

Greenhouses – Heated Facilities

The peak capacity of built greenhouses in the region was achieved in the late 1970s and early 1980s. These standardized greenhouses were a mix of Venlo-type facilities imported from the Netherlands and structures from Bulgaria. However, location selection prioritized their functionality within agricultural production chains rather than considering climatic suitability. Consequently, greenhouses in the northern areas of the country, such as Kumanovo, Chashka, and Prilep, were inherently unsustainable from an economic perspective.

Greenhouses located in regions with modified Mediterranean climates, particularly those heated with geothermal waters, have fared better. Some of these facilities remain active, though their capacity has been nearly halved over time. Greenhouse production declined from 216 hectares in 2013 to 150 hectares in 2022, with a corresponding drop in production from 16,900 tons to 8,300 tons.

From an organizational and production standpoint, the recommended size for a modern greenhouse is 1 hectare, with a lifespan of 15 years. However, the older facilities require a minimum organizational area of 6 hectares (divided into four 1.5-hectare blocks with a shared boiler unit). These outdated systems are costly and challenging to maintain. Over time, depreciation has taken a toll: heating systems are inefficient due to salt deposits, structural components are corroded, and thermal insulation is inadequate. The high energy cost—requiring one liter of fuel oil to produce a kilogram of tomatoes—has forced many operators to cease heating their greenhouses or abandon production entirely.

Active heated greenhouse areas have decreased from 225 hectares to 150 hectares and continue to trend downward (Graph 1). Common crops grown during the heating season include tomatoes and cucumbers, while cabbage and gherkins dominate the non-heating season (Graph 2). A few new operators have invested in updated facilities over the last two decades, covering a total area of about 10 hectares. However, the lack of local expertise has led many operators to rely on foreign consultants, often with remote management systems.

Some smaller-scale facilities (e.g., 2 hectares in Kochani) have ceased production, while others operate mobile tunnels or stationary structures made of wood and covered with polyethylene film. These structures are primarily unheated, though some incorporate heating systems using wood as an energy source. In recent years, tens of hectares of more advanced Israeli-type greenhouses with roof ventilation have been introduced, representing the most modern facilities in the region.

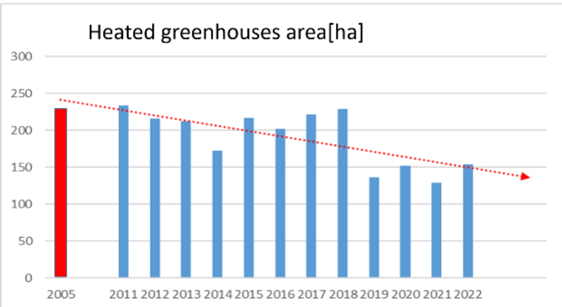


Chart 1. Areas under heated (controlled) conditions in the period (2011-2023) compared to 2005 [ha]

Source: MAFWE Department for vegetable production – latest data from 2023

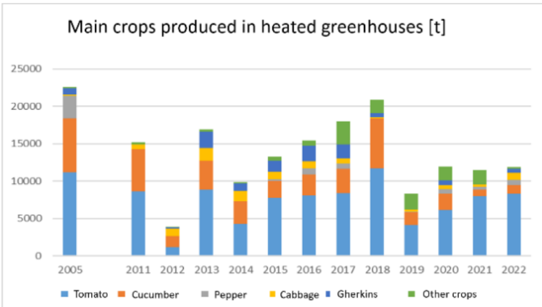


Chart 2. Comparison of crops and harvested quantities under heated conditions in the period (2011-2023) compared to 2005 [t]

Source: MAFWE Department for vegetable production – latest data from 2023

PE tunnels

Greenhouse production has increasingly shifted toward industrial peppers (capiya), which have largely replaced outdoor cultivation. Tomatoes, once the leading greenhouse crop, are losing ground to cabbage. Production methods remain extensive and lack significant technological advancements. In addition to peppers, common crops include cucumbers, onions, garlic (for green consumption), salads, radishes, and other vegetables, primarily for the local market.

Despite the availability of advanced agricultural technologies globally, local plastic house production has seen limited adoption beyond basic micro-irrigation systems. Foil coverings are replaced only when their optical properties degrade, further reducing yields. Between 2013 and 2022, production in tunnels and greenhouses varied, peaking at 6,635 hectares in 2016 before settling at 5,100 hectares in 2022, with a slight upward trend. Annual production under films and greenhouses averages approximately 200,000 tons (Caccamisi et al., 2024).

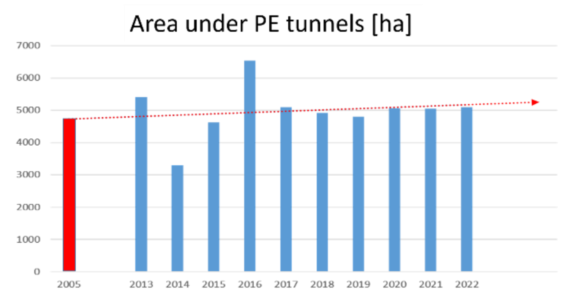


Chart 3. Areas under PE Tunnels in the period (2011-2023) compared to 2005 [ha]

Source: MAFWE Department for vegetable production – latest data from 2023

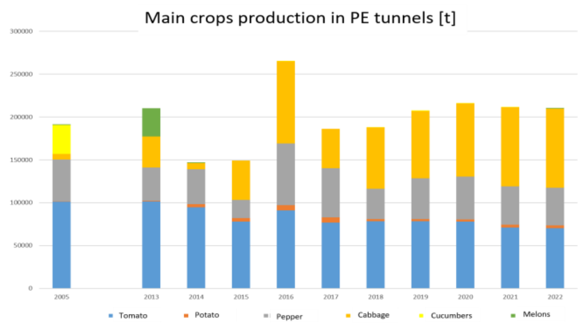


Chart 4. Comparison of crops and harvested quantities under PE tunnels in the period (2011-2023) compared to 2005 [t]

Source: MAFWE Department for vegetable production – latest data from 2023

While these facilities are ideally suited for profitable fresh-market vegetables like tomatoes, cucumbers, and peppers, industrial crops such as cabbage are increasingly cultivated, reducing overall profitability.

With improved microclimate control, greenhouses can maintain stable growing conditions even during extreme outdoor weather, enabling the extension of harvest periods and the cultivation of crops outside their traditional growing seasons. This seasonal flexibility enhances market opportunities and ensures a steady supply of fresh produce. This is particularly significant for the Strumica region, where the production cycles under PE tunnels and in open fields often overlap.

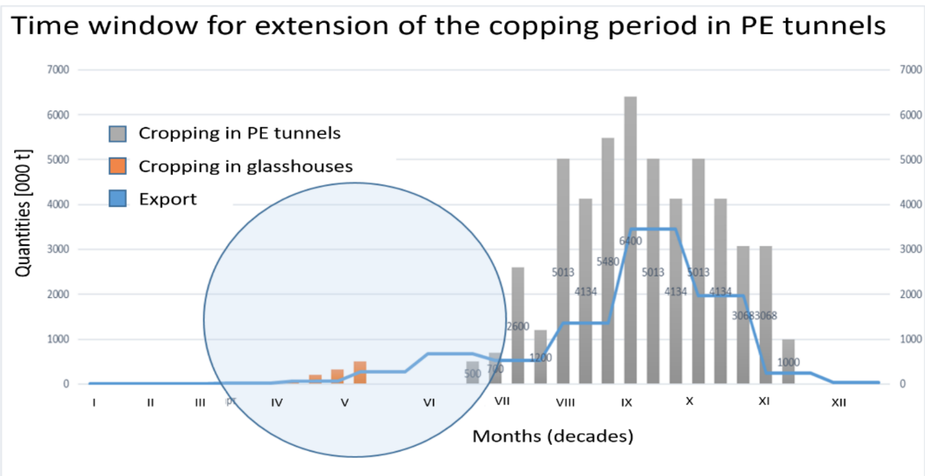


Chart 5 Possibility of extension of the tomato harvest period under protected areas (t)

Source: Popsimonova in (Caccamisi et al., 2024)

Implementing these measures could optimize the timing of greenhouse production, which would be followed by production under PE films (the dominant production method) and ultimately complement outdoor cultivation (Graph 5). Moreover, this approach would encourage the return of high-value cash crops such as tomatoes, cucumbers, zucchini, hot peppers, and similar crops to protected areas, enhancing their profitability and market appeal.

In North Macedonia, the predominantly continental climate, characterized by hot summers and cold winters, poses challenges for maintaining stable microclimatic conditions in greenhouses. To address these challenges, the following practices are recommended:

1. Integration of Microclimate Control Systems - North Macedonia benefits from high solar radiation, which can be harnessed for passive heating. Greenhouses oriented north-south and built with materials capable of absorbing and storing solar energy can significantly lower heating costs during winter (Liang et al., 2017). Investing in high-quality insulating materials for greenhouse structures can reduce heat loss in winter and maintain cooler conditions in summer. Additionally, energy-efficient heating and cooling systems can lower operational costs and support sustainable production (Gorjian et al., 2021). Advanced systems that integrate temperature, humidity, lighting, and ventilation controls into a unified platform can optimize greenhouse conditions. Sensors and automated controllers ensure real-time monitoring and regulation of parameters, improving efficiency and growing conditions (Zainali, 2024). Zhang et al. in 2020 examined the application of IoT technologies to improve water management in high tunnel vegetable cultivation systems. By integrating LoRaWAN, a low-power wide-area network, with soil moisture sensors and irrigation control systems, the research demonstrated a novel approach to precision irrigation. The study highlighted IoT's potential to enhance resource efficiency in vegetable production, reduce labor requirements, and minimize water wastage. Future improvements in sensor accuracy, battery life, and communication reliability will further optimize the technology for broader agricultural applications.

Effective ventilation is crucial for managing temperature and humidity, particularly during the hot summer months. Automatic systems for window operation and fan control ensure optimal air circulation, preventing overheating and condensation, which can negatively impact plant growth (Hung et al., 2023). The research carried out by Sharaf-Eldin et al., (2023) concludes that employing renewable energy-powered systems, such as solar-driven evaporative cooling and fogging, offers a sustainable and cost-effective solution for small-scale farmers. These systems improve the microclimate in walk-in tunnels, enhance crop resilience to heat stress, and significantly increase productivity and fruit quality during challenging growing seasons.

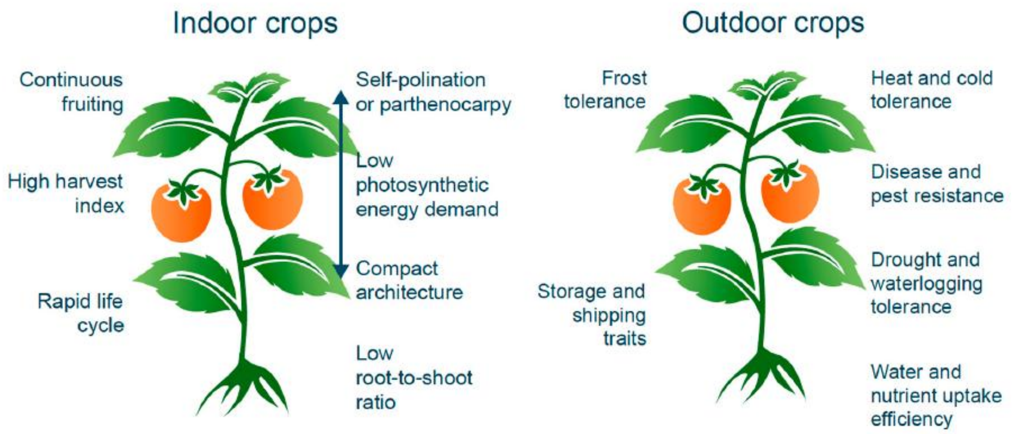
2. Use of Dual-Purpose Mulch - Mulching helps control humidity by preventing evaporation from the soil surface, while its primary purpose remains weed suppression. Murad et al. (2018) demonstrated that carefully controlled low tunnel designs, particularly with the use of double-layer covers and soil mulch, can significantly improve vegetable yields. This approach is especially relevant for regions with challenging climates, offering a sustainable solution to enhance agricultural productivity. Although plastic mulch has shown effectiveness (Bogevska et al., 2021), its environmental impact necessitates exploring biodegradable or reusable alternatives (Khalid et al., 2023).

3. Application of Biological Control for Diseases and Pests - Biological control methods use natural predators or parasites to manage diseases and pests, minimizing reliance on chemical pesticides. This approach improves the health of plants and produces while reducing environmental impact. For example, Push-pull technology involves using plants or substances that repel pests ("push") and others that attract them to trap crops ("pull"). These companion plants provide habitat and nourishment, enhancing the effectiveness of natural pest predators (Legaspi et al., 2024). The control of the microclimate can also contribute to better control of the pests and disease. In a recent study Nitzan et al., (2024) have tested an innovative positive pressure walk-in tunnel for minimizing insect infestation and pesticide use for growing fresh herbs and other leafy vegetables and concluded that the continuous operation of fans within the tunnel not only maintained positive pressure but also ensured consistent air movement, which is crucial for temperature regulation and disease management.

Seedling and Seed Material Challenges

The production of healthy, high-quality seedlings is essential for horticultural success in protected areas. Currently, most farms and individual producers grow their seedlings on-site, dedicating active production areas to this process for one to two months, depending on the crop. Grafted seedlings are rarely used, with a small portion imported from Greece. Grafting offers a viable economic advantage for high tunnel cucumber production, particularly for early-season markets. However, the adoption of this technology depends on factors such as access to skilled labor, cost of grafting materials, and market prices. Future research should explore cost-reduction strategies, such as automated grafting systems, and address challenges related to healing environments and labor availability to expand the use of grafting technology in vegetable production (Rodriguez Izaba et al., 2021) and (Brar et al., 2020).

Producers rely entirely on imported seeds due to the absence of local seed breeding programs. Choice of cultivars, particularly for hybrids, is based on previous experience, market demand, and risk management strategies, such as planting multiple varieties to mitigate issues related to pests, diseases, or market fluctuations. Hybrid seeds for greenhouse production are expensive, sold by the gram or by the number of seeds, and are designed for extended harvest periods.



Graph 6 Desirable traits for fruiting crops grown indoors under controlled-environment conditions relative to crops grown outdoors under field conditions.

Source: Chavan et al, 2022

For instance, indeterminate tomato varieties, capable of fruiting for over a year under suitable conditions, are common in advanced greenhouse systems. However, the limited technology in local greenhouses necessitates the use of determinate varieties, which grow up to five clusters and are more cost-effective. Many producers are unaware of these distinctions, leading to suboptimal seed choices. Similarly, hybrids with resistance to specific pathogens are often selected without consideration of whether those pathogens are prevalent in the region, unnecessarily increasing costs.

Crop cultivars for protected vegetable cultivation differ significantly from those designed for open-field production, as they do not require the same tolerance to a wide range of environmental conditions. In protected cropping, the controlled environment allows for the optimization of specific traits that enhance productivity and efficiency. Developing suitable cultivars for protected cultivation will necessitate a focus on traits such as self-pollination, indeterminate growth, and robust root systems. These traits are particularly advantageous in greenhouse conditions and differ from the characteristics prioritized in outdoor crops, where resilience to fluctuating environmental factors is a key breeding objective (Chavan et al., 2022). Selecting or breeding crop varieties suited to the Macedonian climate can enhance resilience to local challenges, reducing the need for extensive microclimatic interventions and improving overall production success (Savic & Ilin, 2022).

CONCLUSIONS

Macedonia's greenhouse sector has experienced a significant decline in both active areas and productivity. The outdated infrastructure, high energy costs, and lack of modernization have reduced the competitiveness of local production. Many greenhouses, particularly those located in unsuitable climatic regions, have become economically unsustainable. Despite global advancements in greenhouse technology, local producers have adopted only minimal innovations, such as micro-irrigation systems. Key technologies, including automated climate control, advanced heating and cooling systems, and improved structural designs, remain underutilized. This limits the efficiency and profitability of protected vegetable cultivation in Macedonia.

The choice of crops and varieties often does not align with the capabilities of the existing infrastructure or market demands. High-value cash crops, such as tomatoes, cucumbers, and peppers, are losing ground to less profitable crops like cabbage. Furthermore, the reliance on expensive imported hybrid seeds, sometimes unsuitable for local conditions, adds to production costs without proportionate returns.

Macedonia's continental climate, with hot summers and cold winters, imposes significant challenges for year-round greenhouse production. Seasonal extremes necessitate costly heating in winter and cooling in summer, further straining the financial viability of protected cultivation.

Enhanced microclimate management through integrated systems can provide stable growing conditions, extend the harvest season, and enable off-season production. Such advancements can help reintroduce high-value crops into greenhouses and better coordinate production cycles between greenhouses, PE tunnels, and open fields. Macedonia's high solar radiation levels present an opportunity to lower energy costs through passive solar systems which largely untapped due to the limited implementation of suitable designs and materials. However, there is significant potential for revitalizing greenhouse production in Macedonia through investment in modern facilities, automation, and energy-efficient systems. Emphasis on crops with high market demand and the adoption of advanced technologies could improve both yields and profitability.

Practices such as biological pest control, the use of local and adapted crop varieties, and sustainable energy solutions are underdeveloped in Macedonia's greenhouse production. Emphasizing these practices could reduce environmental impacts and align production with global trends in sustainable agriculture. The absence of organized local seedling production forces growers to rely on imported seeds or dedicate valuable production space to seedling cultivation. Developing centralized seedling nurseries could free up space in greenhouses and ensure a consistent supply of high-quality seedlings tailored to local conditions.

A coordinated approach to crop selection and timing could optimize production across greenhouses, PE tunnels, and open fields. This would enable a steady market supply, reduce overlaps in production cycles, and increase overall efficiency.

To secure the future of greenhouse and protected vegetable cultivation in Macedonia, it is essential to address the challenges of infrastructure, technology adoption, crop selection, and environmental sustainability. Implementing targeted investments and modern agricultural practices can unlock the potential for year-round, profitable, and sustainable production in protected areas.

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THE INFLUENCE OF HYBRID VEGETATION LENGTH AND SOWING DENSITY ON MAIZE YIELD

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ABSTRACT

These researches were carried out in 2016 and 2017 on the territory of the municipality of Leskovac. The experiment included 6 maize hybrids different vegetation length (ZP 434, NS 4023, ZP 555, NS 5051, ZP 666, NS 6030) and 3 sowing densities (71428 plants per ha-1; 57142 plants per ha-1 and 47619 plants per ha-1). The experiment was set up according to the randomized block system in 3 replications. Differences in yield between hybrids with a shorter vegetation period, are less pronounced than in cases of hybrids with a longer vegetation period. The length of the growing season of hybrids influenced the differences in yield when different sowing densities were in question. Thus, in hybrids with shorter vegetation (ZP 434 and NS 4023), the highest average yield was in the case of the highest densities, while in hybrids with medium vegetation length (ZP 555 and NS 5051), there were no statistically significant differences in average yield between sowing densities. Hybrids with a longer vegetation period (ZP 666 and NS 6030) achieved the highest yields in the lowest and medium densities and they were significantly higher than the average yields achieved in the highest sowing densities. Considering that lately we have more and more dry years, it is necessary to grow hybrids with defferent length of vegetation, in order to make production safer. In addition, it is absolutely necessary to adapt the sowing density to the length of the hybrid vegetation.

Key words: *Maize, vegetation length, sowing density, yield.*

INTRODUCTION

Maize grain yield depends on several factors. There are three basic groups of factors on which the yield of maize depends, namely the hybrid, climatic conditions of the area, and applied agricultural techniques. The choice of hybrid is of great importance and it is considered that its role in the formation of yield is about 50%. Maize is a robust plant with a long growing season, so it needs a lot of water during the growing season (Bošnjak & Pejić 1997). Rainfall and temperature are of particular importance for maize production. Pejić et al. (2009) point out that maize is most sensitive to water deficit in the soil during the period of vegetative growth, flowering, fertilization, and grain filling. Thus, maize yields under conditions of global warming can be reduced by up to 50 - 80% compared to yields under irrigation conditions (Božić et al., 2007). Lalić et al. (2013) point out that the climate in Serbia will continue to be suitable for agricultural production, while the lack of precipitation will be one of the limiting factors for agricultural production. If the assumptions about future global warming come true, it is predicted that there may be a drop in maize yield by 20-40%, that is, 40-60% with a temperature increase of 2, i.e. 4 0C (Tigchelaar et al., 2018). Sowing density, as well as plant composition, is very important for the formation of maize yield, but only up to a certain limit. Thus, Petrović et al. (2000) point out that the yield of maize grains, especially of early hybrids, increases to a density of about 79,000 plants per ha-1. Similar results were obtained by Mandić et al. (2016), where they emphasize that with an increase in density beyond a certain limit, the grain yield per cob and mass of 1000 grains decreases.

Zivanović et al. (2005) point out that crop density depends significantly on moisture during the growing season, so in dry years grain yield can be reduced by 30%. Due to their smaller habitus, early hybrids are sown in higher densities than late hybrids. However, it should be noted that in years with more precipitation, the highest yields will be in the highest densities, while in dry years the yield will be highest in the lowest densities (Jaramaz, 2015).

These researches aimed to analyze the yields of different maize hybrids depending on the sowing density and favorable climatic conditions of the year.

MATERIAL AND METHODS

The researches were carried out during 2016 and 2017 in the territory of the municipality of Leskovac, on the Smonica soil type. The pre-crop was winter wheat. The experiment included 6 maize hybrids (ZP 434, NS 4023, ZP 555, NS 5051, ZP 666, NS 6030) and 3 sowing densities (71428 plants per ha-1; 57142 plants per ha-1 and 47619 plants per ha-1). The experiment was set up according to the randomized block system in 3 replications. Sowing was done in the middle of April. Standard agricultural technology, characteristic of the given area, was used during the experiment. Soil preparation included autumn plowing to a depth of 30 cm, during which 300 kg ha-1 NPK (16:16:16) was introduced, while the pre-sowing preparation was carried out with a seed drill. Standard agrotechnical measures, characteristic of maize production, were applied. Fertilization was carried out at the 3-5 leaf stage with the use of 200 kg ha-1 KAN. Protection against seed weeds was carried out the day after sowing, with Basar and Rezon, while protection against broad-leaved and narrow-leaved weeds was carried out with Siran and Maton. The yield was calculated and reduced to 14% moisture and statistically processed by analysis of variance using WASP 1.0 software.

Climatic and soil characteristics

Table 1 shows the total monthly precipitation and average monthly temperatures during the maize-growing season. The total amount of precipitation during the growing season in 2016 was 356.8 mm. It should be noted that 207 mm of precipitation fell in the months of June, July, and August, which is very important for the process of forming the maize yield. If we add to this that the average temperatures were also favorable (during the maize gr the importance of climatic conditions, especiallyowing season they were 18.8 degrees Celsius), it can be considered that this year was favorable for maize production.

The total amount of precipitation during the vegetation period in 2017 was 244 mm, which is 112.8 mm less than in 2016. In the same year, during June, July, and August, there was only 73 mm of precipitation (134 mm less than in 2016), so this year can be considered less favorable for maize production. The average temperatures during the maize growing season were 19.2 degrees Celsius and were higher than the average temperatures per month compared to 2016.

Compared to the multi-year average (210 mm), both years had a higher amount of precipitation and approximately the same average air temperature. Total precipitation during the growing season was higher by 146.8, or 34 mm, compared to the multi-year average. This is especially true for rainfall in critical months such as June, July, and August. The average temperatures during the growing season, in both years, compared to the multi-year average, were similar.

The data of Maitah et al. (2021) emphasize temperature, and precipitation, and they point out that yield is negatively correlated with temperatures in July and August and positively correlated with precipitation in the same period.

Table 1. Precipitation (mm) and mean temperatures (0C) in Leskovac

	Apr.	**May**	**June**	**July**	**Avg.**	**Sep.**	**Apr./Sep**
The 2016 growing season							
mm	24.20	69.60	63.00	114.00	30.00	56.00	356.8
^{0}C	13.73	14.70	21.90	22.80	21.40	18.7	18.8
The 2017growing season							
mm	69.00	82.00	19.00	34.00	20.00	20.00	244.0
^{0}C	11.30	16.70	21.90	23.50	23.30	18.70	19.2
Multi-year average 1985-2014							
mm	48	46	37	25	24	30	210
^{0}C	12.5	16.5	19.5	22.0	22.5	18.0	18.5

Table 2. Chemical properties of the soil

Type of soil	**pH**		**Humus**	**Nitrogen**	**Available (mg/100g of soil)**	
	H_2O	KCl	(%)	(%)	P_2O_5	K_2O
Smonica-Vertisol	6.77	5.89	2.18	0.15	20.5	27.3

Soil acidity was determined by the Kapen method, humus was determined by the Kotzman method, total nitrogen by the Kjeldahl method, and available phosphorus and potassium by the Engner-Riehm Al method.

According to the pH values in KCl (5.89), the soil belongs to the group of moderately acidic soils. According to the content of humus in the arable layer (2.18), the soil belongs to the group of low humus soils (Škorić, 1991). According to the content of total nitrogen (0.15), the soil is moderately provided with this element. The phosphorus content of 20.5 mg/100g shows that the soil is optimally supplied with this element. Also, the potassium content of 27.3 mg/100g indicates the optimal provision of this element. Although these soils belong to the group of potentially fertile soils, their intensive use generally requires the application of amelioration measures.

RESULTS AND DISCUSSION

The composition of plants is one of the elements on which the future yield depends. Thus, Sadras and Calderini (2009) note that a good set of plants is the main barrier to the presence of weeds in corn. One of the important factors that determine the density of sowing is the amount and distribution of precipitation. Starčević and Latković (2005) consider that the optimal density is variable and should be adapted to the year conditions, as well as the area.

The following table shows the yield of different maize hybrids achieved in two years that had different climatic conditions for production.

The average yield of maize for all years and densities ranged from 9.66 t ha-1 when the hybrid NS 4023 was in question to 11.54 t ha-1 in the case of the hybrid ZP 555. The hybrids ZP 555 and NS 5051 had the highest average yields, which were significantly higher than the yields of hybrids ZP 434 and NS 4023. There were no significant differences in the average yield between the hybrids ZP 434 and NS 4023, nor between the hybrids ZP 666 and NS 6030, that is, the hybrids ZP 555 and NS 5051.

The effect of the year on the average yield was very pronounced, which can be attributed to the influence of more favorable climatic conditions, especially the distribution of rainfall in 2016. The total amount of precipitation during the growing season in 2016 was 112.8 mm higher than in 2017. In the same year, in the months of June, July, and August, there was 134 mm more than in 2017, so 2016 can be considered much more favorable for maize production.

Table 3. Yield maize (t ha-1) depending on year and sowing density

A. Hybrids	B. Years	C. Sowing density			Average AB
		G1	G2	G3	
ZP 434	2016	12.48	11.94	11.04	11.82
	2017	8.85	8.02	7.49	8.12
	Average AC	10.66	9.98	9.26	9.97
NS 4023	2016	12.03	11.20	10.43	11.22
	2017	8.56	8.03	7.72	8.10
	Average AC	10.29	9.61	9.08	9.66
ZP 555	2016	14.26	14.46	13.15	13.96
	2017	8.73	9.08	9.55	9.12
	Average AC	11.49	11.77	11.35	11.54
NS 5051	2016	13.91	13.65	13.12	13.56
	2017	8.03	8.39	8.41	8.28
	Average AC	10.97	11.02	10.76	10.92
ZP 666	2016	12.98	14.10	13.93	13.67
	2017	7.16	7.81	8.01	7.66
	Average AC	10.07	10.95	10.97	10.66
NS 6030	2016	12.73	13.68	13.21	13.21
	2017	6.92	8.26	8.84	8.00
	Average AC	9.82	10.97	11.03	10.60
	Average C	**10.55**	**10.72**	**10.41**	**10.6**
	LSD	A	B	C	
	0.05	0.647	0.374	0.458	
	0.01	0.859	0.496	0.608	

Thus, with all hybrids for all sowing dates, the average yield in 2016 was significantly higher than in 2017 (Graph. 1). Yield reduction depending on the favorable climatic conditions of the year ranged from 38.4% (NS 4023) to 78.4% (ZP 666) and depended on the length of the hybrid's vegetation. So, hybrids with shorter vegetation had an average yield reduction of 41.9%, hybrids with medium vegetation length of 58.4%, and hybrids with a longer vegetation period decrease of 71.7%. These results are in agreement with the statements of Bella et al. (2014) who point out that the amount and distribution of precipitation during the growing season, especially during flowering and grain filling, play an important role in the manifestation of the fertility potential of hybrids. Bassey et al. (2019) indicate the importance of the amount and distribution of precipitation during panicle formation, fertilization, and grain filling. Not only the lack of precipitation but also higher summer temperatures and how they affect the yield, so that it can be reduced by up to 47% (Lui et al., 2019 Lobell and Burke, 2010).

If we look at the average yield for all hybrids and years, there were no significant differences in sowing densities. However, if we look at the average yields for both years, the length of the hybrid's vegetation affected the yield differences when the sowing densities are in question (Graph 2). Thus, in the case of the first two hybrids, which have a shorter growing season, the highest average yield was at the highest densities, and it significantly decreased until the lowest sowing density. There were no statistically significant differences in the average yield between sowing densities in hybrids with a medium vegetation length (ZP 555 and NS 5051).

Hybrids with a longer vegetation period (ZP 666 and NS 6030) achieved the highest yields in the lowest and medium densities and they were significantly higher than the average yields achieved in the highest sowing densities.

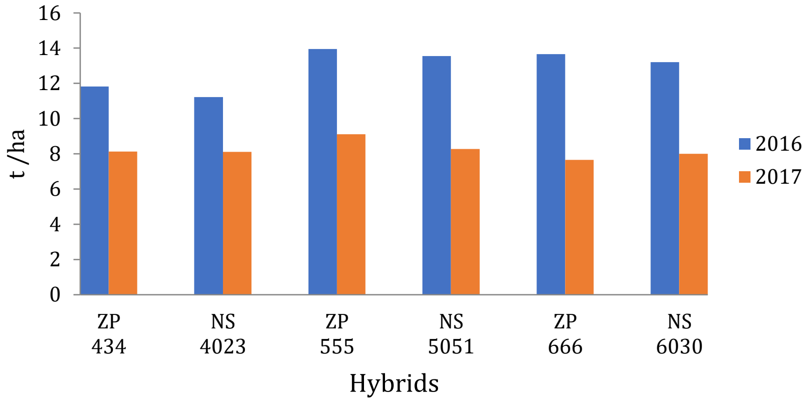

Figure 1. Yield maize of some hybrids (t ha-1) depending on the year

Solomon et al. (2017) point out that hybrids with a longer vegetation period are at greater risk of yield reduction at higher densities, which was also the case in our research. Hybrids with a short growing season have a smaller habitus and smaller requirements for living space, so the highest yields are logical in the highest densities, unlike hybrids with a longer growing season. These results are in accordance with the statements of Mandić et al. (2016) who emphasize the importance of the optimal assembly for each hybrid because only then do the plants achieve the highest yields. Also, Barakatullah and Safdary (2021) point out that the sowing density should be adapted to the sowing time and the hybrid in order to achieve the highest yields. The highest yield of 14,46 t ha-1 was achieved by hybrid ZP 555 in 2016 at the medium seeding density, while the lowest yield of 7.49 t ha-1 was achieved by hybrid NS 434 at the lowest seeding density in 2017.

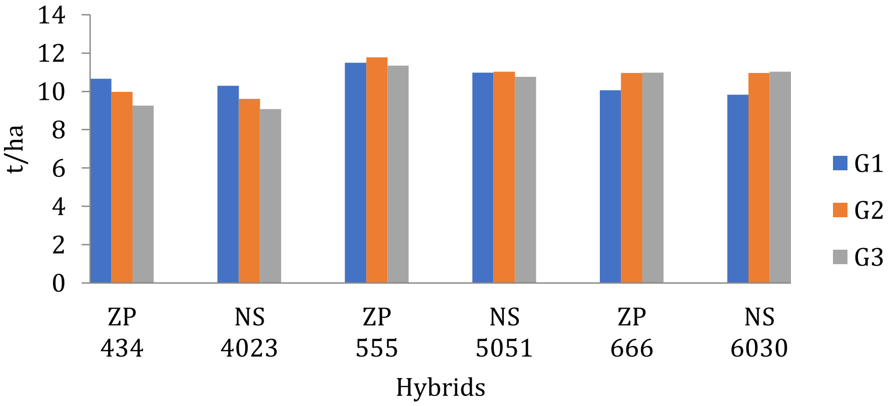

Figure 2. Yield maize of some hybrids (t ha-1) depending on the sowing density

It is evident that in climatically favorable years, hybrids with a shorter vegetation period show their genetic fertility potential, while in climatically unfavorable years, hybrids with a shorter vegetation period prove to be safer in terms of yield. Therefore, it is recommended to sow several hybrids of different vegetation lengths and adjust the sowing density to the hybrid, so that the production is safer and more stable.

CONCLUSION

The effect of the year on the maize yield was very pronounced, so 2016 was significantly more favorable for maize production compared to 2017. The decrease in yield depending on the favorable climatic conditions of the year ranged from 38.4% (NS 4023) to 78.4% (ZP 666) and it depended on the length of the hybrid's vegetation. Thus, hybrids with a shorter vegetation period had an average decrease in the yield of 41.9%, hybrids with an average length of vegetation of 58.4%, and hybrids with a longer vegetation period of 71.7%. The average yields for both years show that the length of the growing season of the hybrid influenced the yield differences when the sowing densities were in question. Hybrids with a shorter growing season (ZP 434 and NS 4023), achieved the highest average yield at the highest densities, but it decreased significantly up to the lowest sowing density. There were no statistically significant differences in the average yield between sowing densities in hybrids with a medium vegetation length (ZP 555 and NS 5051). Hybrids with a longer vegetation period (ZP 666 and NS 6030) achieved the highest yields in the lowest and medium densities and they were significantly higher than the average yields achieved in the highest sowing densities. In years favorable for production, such as 2016, hybrids with a longer growing season showed their genetic potential for yield and were more dominant than hybrids with a shorter growing season. For this reason, for the examined area, if maize is grown in dry farming, it is recommended to sow several hybrids of different vegetation lengths and adjust the sowing density to the hybrid, so that the production would be safer.

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MORPHOLOGICAL TRAITS AND IMPACT OF HARVEST TIME ON THE HEAD RICE (*ORYZA SATIVA* L.) YIELD

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ABSTRACT

In this study 13 introduced genotypes were compared with two genotypes used as standards. Seven are Italian varieties, while six are Turkish. The experiment was conducted in 2022, using a randomized block design with 4 replications in Kochani rice production region. Examined parameters were: number and length of productive panicles/m^2, grain yield/m^2, 1000 grain mass, hectoliter mass and head rice yield from three consecutive harvest terms regarding seed moisture. The obtained results were processed and analyzed through variance analysis and tested with the LSD test. The variance analysis of the different genotypes for morphological and production traits showed that all genotypes have statistically significant differences for all examined properties. Genotypes 8 and 11 demonstrated the highest values for the number of productive panicles/m^2, grain yield/m^2 and hectoliter mass but the lowest values for 1000 grain mass. Genotype 15 had the longest panicles and the greatest 1000 grain mass, but the lowest number of productive panicles/m^2. Regarding the yield parameter all genotypes had the highest yield in the first harvest term while the yield decreased as the harvest time was extended. The highest head rice yield in the first harvest term recorded Genotype 1, while for the second and third harvest term showed Genotype 12. The lowest yield for all three harvest terms was noted in Genotype 9. Following the results, these examined genotypes can be included in breeding programs, as selection process plays a crucial role in enhancing of the genetic potential by introducing high-yielding and quality rice varieties.

Key words: *Head rice yield, grain yield, 1000 grain mass, productive panicles, panicle length.*

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial cereal crop globally, contributing to 27% of total cereal production (Huang et al., 2011). To meet this demand, global rice production must increase by over 1.2% annually (Normile et al., 2008). However, there is limited potential for expanding rice cultivation areas in the future. Key factors in rice production include yield, harvest index, and quality (Andreevska et al., 2015). Rice yield is influenced by several factors, such as the number of productive panicles/m2, the percentage of grain filling and the 1000 grains mass. Yield can be enhanced by increasing the number of panicles/m^2 or the number of grains in the panicle, or both (Yoshida et al., 2006). However, enhancing these two components is challenging due to their compensatory nature (Peng et al., 2006). Additionally, the effectiveness of yield components can vary across different environments (Katsura et al., 2008; Ying et al., 1998). Although rice is not a primary food for the population in North Macedonia, it holds significant economic value as a cereal crop, providing stable and high yields (Andov and Andreevska, 2010). A high yield of paddy rice does not necessarily correlate with a high yield of white rice (Andov et al., 2003, Andov et al., 2008/2009, and Ilieva et al., 2009). To achieve optimal yields of both paddy and white rice, the timing of sowing and harvest, as well as post-harvest management, is essential (Ilieva et al. 2014). Optimal moisture content at harvest is between 18% and 20% (Bautista et al. 2009; Ilieva et al. 2009). For storage, paddy rice should be dried below 14% grain moisture, and rice seed material to below 12%. The ideal moisture content for paddy rice milling is between 12-14% (IRRI). Higher moisture content leads to increased losses from poor grain quality, while lower moisture content results in more grain breakage. Thus, the aim of this research is to evaluate new rice varieties in the Kochani region, focusing on those well-suited to local conditions for better productivity, yield and quality, supporting sustainable rice farming. The findings will be used to preserve these valuable genetic resources, further incorporate them into rice breeding programs and ensure their sustainable use.

MATERIAL AND METHODS

As plant material for this research, 15 genotypes of rice were used. Eight of them are Italian and six of them are Turkish. As standard genotypes were used one Italian and one Macedonian variety. The field experiment was set up during 2022 in Kochani, the main rice-producing region of Macedonia and represents typical rice growing conditions in the country. Standard agrotechnology for rice production was applied. The experiment was set up in a randomized block design, with 4 replications, with a total plot area of 10 m^2. At the end of the vegetation period, at the time of full maturity of the rice, seed samples were collected manually from each replication and from each variety. On the same day, the moisture content of the seed was measured for these samples and later these samples were used to determine the milling yield. Such samples were collected consecutively from 3 different time periods (every 10 days), in order to see from which period, with which seed moisture, the best milling yield was obtained. At the end of the vegetation period, at the time of rice harvest, from each replication and from each variety, by the square method, average samples of one square meter were taken for further laboratory tests. The following analyses were performed in the laboratory: number of productive panicles per m^2, panicle length taken from 10 plants from each replication for each variety, grain yield per m^2, 1000 grain mass, hectolitre mass and analysis of the milling yield from the three consecutive seed moisture terms. From each genotype, from the three consecutive seed moisture terms, the yield of milling rice was determined on a laboratory milling machine. The obtained results were processed and analysed by ANOVA and tested with the LSD test (with a probability level of 0.05 and 0.01).

RESULTS AND DISCUSSION

The analysis of variance of different genotypes for morphological and productive traits is presented in Table 1. From the obtained results, it can be observed that all genotypes showed statistically significant differences for all examined traits. Alam et al. (2014) and Yaqoob et al. (2012) also noted significant variations among genotypes in their studies for the number of productive panicles/m^2, the 1000 grain mass, and grain yield/m^2.

Table 1. Analysis of variance for some morphological and production traits of 15 rice genotype

Mean Sq	DF	PL (cm)	NPT/m^2	GY/m^2 (g)	1000 GM (g)	HM (kg/hl)
Genotype	14	13.83*	127472*	273732*	84.68*	90.74*
Replications	3	3.177	653	8589	2.33	0.84
Error	45	1.054	3905	13079	1.09	1.18

MS - mean of square, DF - degree of freedom, PL - panicle length, NPT- number of productive tillers, GY- grain yield, 1000 GM- 1000 grain mass, HM - hectolitre mass   *shows significance at the 0.001 level

The average values of the 15 rice genotypes for the examined traits are presented in Table 2. The tabular representation shows that genotype 15 has longest panicle (17.18 cm), while genotype 12 has the lowest recorded average value (10.93 cm) for this trait. The number of productive panicles/m^2 directly affects the grain yield in rice and is proportionally dependent on it.

Table 2. Average values of 15 genotypes for various morphological and production traits related to yield

Geno type	PL (cm)	NPT/m^2	GY/m^2 (g)	1000 GM (g)	HM (kg/hl)	HRY 1st (%)	HRY 2nd (%)	HRY 3rd (%)
1	4.15	670.75	1125.50	37.68	40.80	71.40	63.48	57.45
2	5.83	749.75	1068.25	36.60	45.40	68.90	58.93	59.25
3	6.03	513.25	921.50	37.98	50.60	53.20	50.75	47.13
4	2.85	700.75	1009.00	36.50	43.08	68.93	57.28	59.73
5	3.68	841.50	1046.25	30.80	49.30	68.98	61.10	62.10
6	6.08	453	623.25	35.43	46.73	65.95	56.13	59.60
7	5.78	567	453.75	38.50	41.83	65.98	61.15	65.25
8	2.20	935.75	1333.25	24.70	51.45	68.20	63.65	62.50
9	4.43	581.25	684.75	37.48	50.65	52.93	41.30	42.85
10	3.38	762.25	488.00	36.65	33.45	63.28	59.93	64.05
11	1.43	956.50	619.00	26.68	46.93	61.68	61.53	53.13
12	0.93	764.25	927.25	30.83	47.98	70.55	65.98	67.23
13	2.70	669.75	1027.00	33.63	45.70	65.15	61.65	62.05
14	4.73	588.25	887.50	38.78	47.58	56.90	54.10	42.95
15	7.18	292.50	543.00	40.30	49.95	53.15	52.58	45.30
min	0.93	292	453.75	24.70	33.45	52.93	41.30	42.85
max	17.18	956.50	1333.25	40.30	51.45	71.40	65.98	67.23
mean	4.09	669.77	850.48	34.83	46.09	63.68	57.97	56.70
SD	2.048	181.82	275.906	4.581	4.735	7.365	9.522	9.327
$LSD_{0,05}$	0.33	20.09	36.77	0.34	0.35	0.74	0.52	0.53

PL-panicle length, NPT- number of productive tillers, GY-grain yield, 1000GM- mass of 1000 grains, HM- hectolitre mass, HRY 1st, 2nd, 3rd - head rice yield (first, second and third term), SD - standard deviation

Regarding this trait, genotype 11 showed the highest average value (956.50), followed by genotype 8 (935.75), while the lowest average value was recorded for genotype 15 (292.50). Singh et al. (2023), who studied 21 rice genotypes, including hybrid rice in rainy climate conditions, reported highly significant variations for various rice traits, including the number of productive panicles/m^2. The average of 1000 grain mass is 34.83 g, with the genotype 8 having the lowest value (24.70 g), followed by genotype 11 (26.68 g), which is statistically significantly lower than genotype 15 (40.30 g), the variety with the highest recorded average for this trait. Ten out of the fifteen genotypes exceeded the general average for this trait, which is consistent with the findings of Singh et al. (2023) but contradicts the study of Tahir et al. (2002), who stated that 1000 grain mass is controlled by genotypic differences among genotypes. Regarding grain yield/m^2, the lowest average value was observed in genotype 7 (453.75 g), followed by genotype 10 (488 g), while genotype 8 had the highest grain yield/m^2 (1333.25 g). Genotype 8 showed the highest hectolitre mass (51.45 kg/hL), followed by genotype 9 and genotype 3 (50.65 kg/hL and 50.60 kg/hL, respectively). The lowest value for this parameter was recorded in genotype 10 (33.45 kg/hL).

In table 2 and 3 are presented the results about the total yield of white head rice at three different harvest terms. The results from the tabular representation show that all genotypes have the highest head rice yield from the first harvest term, where the grain moisture content was about 23% (Table 3) and with the prolongation of the harvest time and decreasing the grain moisture content, the milling yield reduces. The total white head rice yield at the 2nd and the 3rd harvest terms was similar for both, but significantly lower than that of the 1st harvest term. This decline in milling yield during the later harvests was likely caused by increased grain breakage during processing. These findings align with those of Ntanos et al. (1996) and Berrio and

Cuevas-Perez (1989) for other rice varieties. Their research indicated that delaying harvest by two weeks led to a 3% reduction in total white head rice yield.

Table 3. Total milling yield at three different harvest terms. Means are averaged over 15 genotypes

Harvest time	Grain moisture content (%)	Total yield of white head rice (%)
1st	23.16	63.68*
2nd	20.22	57.97
3rd	17.72	56.70

*Shows significance at the 0.001 level

In the first harvest term, genotype 1 has the highest white head rice yield (71.40%), while the lowest was recorded for genotype 9 (52.93%), which also has the lowest white head rice yield in the second and third harvest terms (41.30% and 42.85%, respectively). The highest white head rice yield for the second and third harvest terms belongs to genotype 12 (65.98% and 67.23%, respectively). The results about the harvest moisture content, processing of paddy rice after harvest, and other factors influencing yield and white rice quality are in accordance with the following researches: Saeed and Mohammad, 2013, Akowuah et al., 2012, Saeed and Mohammad, 2011, Thompson and Mutters, 2006.

CONCLUSION

The results clearly show that all varieties exhibit statistically significant differences for all examined traits, including panicle length, number of productive panicles/m^2, 1000 grain mass, hectolitre mass, grain yield/m^2, and yield of white head rice (from three different harvest terms). Genotype 15 has the longest panicles and the highest 1000 grain mass but the lowest number of productive panicles/m^2. In terms of the number of productive panicles/m^2, genotype 11 showed the best results, along with genotype 8, which also had the highest values for grain yield/m^2 and hectolitre mass. However, genotype 8 recorded the lowest 1000 grain mass, with similar results observed in genotype 11. Genotypes 7 and 10 had the lowest grain yield/m^2, while genotype 10 also had the lowest hectolitre mass. Regarding milling yield, all varieties achieved the highest percentage in the first harvest term, with the yield decreasing as the harvest was delayed. Genotype 1 had the highest white head rice yield in the first harvest term, while genotype 12 recorded the highest values in the second and third harvest term. The lowest white head rice yield across all three harvest terms was observed in genotype 9. The obtained information will be used for the proper conservation of these valuable genetic resources, their further incorporation into breeding programs, and ultimately their sustainable utilization. This is crucial, as the income of rice producers depends on the high productivity of varieties, as well as good milling yield and rice quality.

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DETERMINATION OF GENETIC DIVERSITY BASED ON PHENOTYPIC CHARACTERISTICS IN RICE GENOTYPES (*ORYZA SATIVA* L.)

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ABSTRACT

The aim of research was to assess the diversity of 53 rice genotypes based on 9 phenotypic traits: stem height, panicle length, plant height, panicle mass, number of productive grains in panicle, number of unproductive grains in panicle, productive grains mass, unproductive grains mass and number of primary branches in panicle. According to the results obtained from the examined traits, a principal component analysis was performed and determined to an Eigen-value > 1 for each genotype, followed by cluster analysis. Principal component analysis (PCA) showed that the first two principal components account for 73.19% of the total genetic variance among genotypes. The first principal component accounts for 52.25% of the total variance, in which panicle mass and productive grain mass have the largest contribution. Ward's cluster analysis using squared Euclidean distance, revealed 4 main groups with different numbers of subgroups within each group. This indicates significant genetic diversity among the analyzed genotypes. The UPGMA method was used to construct the dendrogram and the results were processed using the R 3.5.1 software. The obtained results for the examined phenotypic traits indicate that the genotypes are great genetic resources for picking desired traits in rice breeding programs.

Key words: *plant height, panicle weight, productive grain, primary branches*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely cultivated crops in the world; distributed across a variety of climates and regions, it has developed a diverse array of genotypes and phenotypes. In the process of producing new varieties, genetic diversity was greatly reduced in both wild and cultivated rice (Park et al., 2019). In recent years, there have been studies in which rice germplasm has been characterized, for which a wide range of variability has been obtained (Bollinated et al., 2020; Madhubabu et al., 2020). Despite the abundance of rice genetic resources, the genetic similarity of rice cultivars is increasing and species diversity continues to decrease, as only a few specific cultivars are used in rice breeding (Nanda и Sharma, 2003, Das et al. 2013). In order to meet the huge demand for rice grain, development of high yielding genotypes with desirable agronomic traits is necessity. Any crop improvement program depends on the utilization of germplasm stock available in the world. Grain yield is a complex trait which is environmentally influenced and controlled by many genes and their genetic variability (Wu et al., 2015). Yield component directly or indirectly increasing grain yield if the components are highly heritable and genetically independent or positively correlated with grain yield (Hasan et al., 2013). Many researchers applied indirect breeding for yield based on yield components and found more efficient than direct breeding for yield on several crop species (Yuan et al., 2011;

Kumar and Bahl, 1992; Saadalla, 1994). Parents identified on the basis of divergence for any breeding program would be more promising (Shahidullah et al., 2009). Plant breeders usually breed for yield component traits which indirectly increase yield. The relationship between rice yield and its components has been studied widely at phenotypic level (Kwon et al., 2002; Akinwale, 2011; Hairmansis et al., 2010; Sadeghi, 2011). Principal component analysis (PCA) is a multivariate technique commonly used for data compression, reduction, and transformation. Its goal is to extract the essential data from a table of values, represent them as a set of new orthogonal variables, called principal components, and display the similarity pattern as points on a graph (Rahangdale, et al., 2021).

PCA is an analytical method that finds the best representations of the differences of each dataset and distinguishes the data by each element. In other words, PCA is a method where data are presented as axes. The axis with the greatest variance is set as the first main component, and the axis with the second largest variance is displayed as a diagram (Park et al., 2019). Further, PCA identifies the minimum number of components, which can explain maximum variability out of the total variability (Anderson, 1972; Morrison, 1982) and also to rank genotypes on the basis of PC scores. Considering the importance of principal component analysis and cluster analysis for genotype classification, the main objective of this research was to analyze the phenotypic characteristics of 53 rice genotypes, to obtain precise information about the variability of the studied germplasm and to classify the rice genotypes.

MATERIALS AND METHODS

A field experiment with 53 rice genotypes of foreign and domestic origin was set up in 2022, on an experimental plot of 3m^2, in a randomized block design, in three replications. Standard agronomic techniques for rice production were applied before and during the vegetation period. From each genotype, 10 plants were randomly selected and nine phenotypic traits were examined for each of them: plant height, stem height, panicle length, panicle mass, number of filled grains per panicle, number of unfilled grains per panicle, filled grains mass, unfilled grains mass and number of primary branches per panicle. In order to determine the phenotypic properties of the rice plant stems and panicles, which have the greatest impact on the total variability of the analyzed germplasm, a principal component analysis (PCA) was performed. To determine the genetic diversity of the different rice genotypes, the principal components were determined to an Eigen-value > 1. The construction of the dendrogram was made based on Ward's cluster analysis, using squared Euclidean distance, the UPGMA method was used to construct the dendrogram. The obtained results were processed using the R 4.3.1 2021 software.

RESULTS AND DISCUSSION

Among the most important elements of any successful breeding, especially for rice as a self-fertilizing crop, is the study of genetic variation. By analyzing genetic diversity, breeders can improve existing and create new, high-yielding genotypes, by selecting optimal materials for further genotype improvement and effective management of rice genetic resources (Rezk et al., 2024). There are many rice varieties that possess desirable characteristics that can be exploited in breeding programs. Therefore, it is of great importance to make an accurate description and assessment of variability. Characterization of germplasm is essential for the conservation, protection and sustainable use of plant genetic resources (Sparato and Negri, 2013; Rivera et al., 2016). Out of five, two principal components were extracted with an Eigen-value greater than 1 and a total variance of 73.19%, for nine examined traits. The first principal component (PC1) explains 52.25% of the total variability, which is due to all traits, except for the number of unfilled grains in the panicle and for the number of primary branches, while the second principal component (PC2) explains 20.94% of the total variability (Tab. 1.). Shoba et al., 2019, out of nine principal components, obtained four with an Eigen-value greater than 1 and a total variability of 70.14%, for nine traits, in 67 rice germplasm lines.

Table 1. Results of principal component analysis (PCA) for phenotypic traits in rice genotypes

Phenotypic traits	PC1	PC2
Stem height	0,795	-0,446
Panicle length	0,802	-0,167
Plant height	0,824	-0,424
Panicle mass	0,922	0,094
Number of filled grains per panicle	0,831	0,309
Number of unfiled grains per panicle	-0,132	0,760
Filled grains mass	0,915	0,084
Unfilled grains mass	0,343	0,614
Number of primary branches	0,480	0,640
Eigen-value	4,70	1,88
Variance (%)	52,25	20,94
Cumulative variance (%)	52,25	73,19

According to the characteristics of the stems and panicles, the studied genotypes were classified into 4 main groups (Fig. 1). Genotype 152, from the third group, stands out according to the height of the stem, the height of the whole plant and the number of primary branches. Genotypes 104 and 119, also from the third group, are characterized by the highest values for panicle length, panicle mass, number of filled grains per panicle, as well as filled grains mass per panicle. Many previous studies have shown a positive correlation between grain yield and yield components, such as panicle length and the number of primary branches per panicle (Gautam and Shrestha, 2023; Mei et al., 2016; Crowell et al., 2016, Wang et al., 2009; Li et al., 2003; Sarwar and Ali, 1998). The genotypes from the first and second groups have the lowest values for most of the studied traits, except for number of unfilled grains per panicle, where five genotypes (145, 151, 139, 121, 117) from these two groups have the highest value for the examined traits.

Cluster analysis allows the separation of groups of genotypes with similar characteristics (Nick et al., 2008), which can be used to pick desirable parents for crossing. If the crossing is between varieties, within the same group or a closely distant group, there is a possibility of obtaining smaller variations, while crossing between distant groups will give a larger variation. Based on the determined genetic distance, according to phenotypic traits, 4 main groups were detected, each of which consists of a different number of subgroups (Fig. 2). Cluster I is divided into two subgroups and consists of genotypes with the lowest average values for the following traits: stem height (61.15 cm), panicle length (12.82 cm), plant height (73.97 cm), panicle mass (1.75 g), number of filled grains per panicle (48), filled grains mass per panicle (1.48 g). Cluster II is the largest and contains 20 genotypes characterized by the lowest average number of unfilled grains per panicle (14.89). In cluster III, genotypes are grouped based on the highest average values for all 9 examined traits, and they are significantly distinguished by stem height (99.73 cm), plant height (116.49 cm), panicle mass (3.21 g), number of filled grains per panicle (90.37), filled grains mass per panicle (2.88 g) and number of primary branches (10.65). (Table

2). Genotypes belonging to cluster IV have medium-high values for stem height, panicle length and plant height.

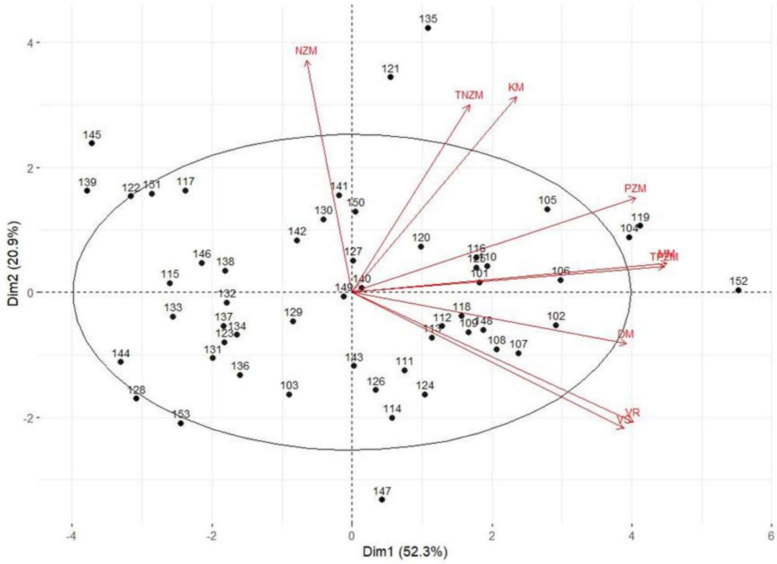

Figure 1. Biplot of principal components (PC) for the analyzed genotypes and phenotypic traits

Genetic similarity or genetic distance between genotypes, except in rice (Touhiduzzaman et al. 2016; Tiwari et al., 2013; Stanisz, 2007), is also shown with a dendrogram in gerbera (Sikder et al., 2015), tomato (Hossain et al., 2015) and cassava (Mehraj et al., 2014).

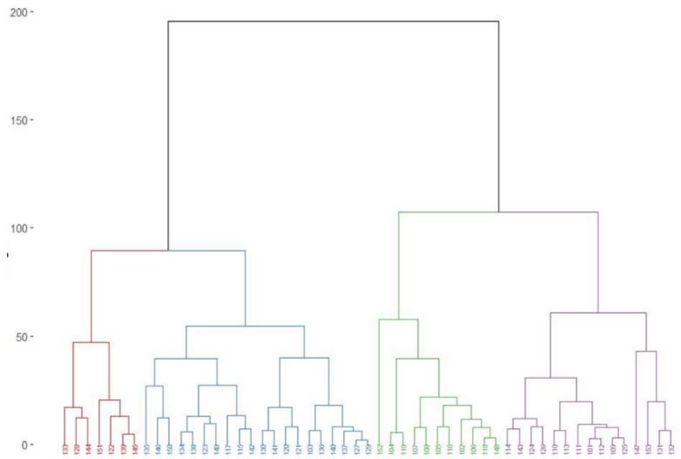

Figure 2. Cluster analysis of 53 rice genotypes

The obtained results show a certain degree of genetic diversity of the characterized rice genotypes for the analyzed traits. The performed analyses proved to be effective in detecting differences between genotypes, in terms of phenotypic traits of rice. Multivariate techniques for classifying genotypes based on their genetic divergence have been used in a number of studies (Sandeva Atanasova et al., 2021; Ridzuan et al., 2019, Bianchi et al., 2016; Rivera et al., 2016; Jankulovska et al., 2014).

Table 2. Average, minimum and maximum values for the analyzed phenotypic traits by clusters

Cluster	Value	Stem height (cm)	Panicle length (cm)	Plant height (cm)	Panicle mass (g)	No. of filled grains per panicle	No. of unfilled grains per panicle	Filled grains mass per panicl e	Unfilled grains mass per panicle	No. of primary branches
Cluster I	Mean	61,15	12,82	73,97	1,75	48,00	21,99	1,48	0,27	9,32
	Min	51,73	11,13	65,87	1,61	36,70	5,00	1,27	0,22	7,07
	Max	71,07	14,13	82,20	2,08	52,63	34,27	1,81	0,35	12,63
Cluster II	Mean	67,10	13,81	80,91	2,62	73,42	14,89	2,30	0,32	9,99
	Min	56,13	11,00	68,42	2,06	65,70	8,50	1,84	0,17	8,37
	Max	78,57	16,48	92,75	2,96	83,37	22,30	2,64	0,40	11,73
Cluster III	Mean	99,73	16,76	116,9	3,21	90,37	16,40	2,88	0,33	10,65
	Min	90,70	15,63	106,3	2,81	71,90	10,43	2,47	0,30	8,87
	Max	122,87	19,12	141,8	3,79	105,67	24,80	3,43	0,36	15,33
Cluster IV	Mean	90,12	15,33	105,45	2,30	54,00	16,36	1,93	0,28	8,59
	Min	77,83	13,83	92,47	1,29	40,47	8,43	1,10	0,19	6,97
	Max	105,40	17,00	122,40	3,07	71,67	22,60	2,69	0,38	11,77

CONCLUSION

Based on the analysis of the genetic distance of 53 rice genotypes, through the characterization of nine phenotypic traits, using multivariate techniques, it has been determined that there is a certain diversity of the studied germplasm. The results obtained clearly distinguish the most different and the most similar rice genotypes. According to the results and discussion of the current study, it can be concluded that different phenotypic traits, related to the growth and yield of rice, have a mutual relationship.The analysis of phenotypic traits in different genotypes and the determination of their diversity, can significantly contribute to the further rice breeding.

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EFFECTS OF CALCIUM SPRAYS ON YIELD AND GRAIN QUALITY OF MAIZE (*ZEA MAYS* L.)

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ABSTRACT

This work aimed to evaluate the effect of calcium as the main component in foliar fertilizer Megagreen, on corn yield at hybrid ZP 677 (FAO 600) and Mn, Zn and Fe content in corn grain. The experiment has been designed by the method of random block system with 4 treatments and 3 repetitions. Treatments consisted three levels of the fertilizer with in a concentration of 0.3; 0.6 and 0.9 percent of fertilizer Megagreen and control variant (without fertilizing). The three concentrations of fertilizer were applied four times during the growing period, starting from the stage of 7-8 leaves (V7) in an interval of 10-15 day. The experiment was carried out on experimental site with alluvial soil in Skopje region, during two years.

During the harvest, the samples of corn grain from each variant were collected for chemical analysis. The results showed that the average highest yield (10.1 t/ha) had the variant treated with Megagreen in concentration of 0.6%, while the lowest yield was recorded at control variant (9.5 t/ha). The statistical analysis showed significant positive effect of foliar application in concentration of 0.9% on the content of Mn (5-6mg/kg) and Zn (22-26.33 mg/kg) in corn grain. The results revealed that foliar spray of Ca did not significantly affect the concentration of Fe (23.5-25.66 mg/kg) in the grain.

Key words: *Megagreen, fertilizer, spray, corn, grain*

INTRODUCTION

The foliar supply of nutrients is recognized as one of the most efficient agronomic practices to alleviate nutritional deficiencies in plants caused by insufficient soil availability (Krishnasree et al. 2021). The benefits of this practice stem from the improved nutrient uptake as compared to conventional soil-applied fertilization and the possibility of applying such a practice during critical stages of plant growth (Fernández and Brown, 2013), regardless of soil moisture and drought conditions (Visioli et al., 2018). Foliar application of nutrients allows small quantities of target elements, tailored to the specific requirements of the particular crop species at different growth stages, to be supplied directly to the plant. Adequate concentrations of nutrients can improve plant nutritional status, stimulate root growth, and enhance mineral absorption from the soil solution (Niu et al. 2021). Macro-nutrients applied through foliar spraying can directly penetrate the leaf through the external leaf cuticle or enter through the stomatal openings. To date, foliar supply of nutrients and biostimulants has been little explored in maize, often focusing on low input management systems in developing countries (Afe et al., 2015; Fall et al., 2023). However, foliar application of nutrients and biostimulants during critical phenological stages and plant conditions has great application potential in intensive agricultural systems as well. In such systems, their application can counteract nutritional deficiencies and improve growth and development (Girma et al., 2010). The chemical composition and morphology of maize leaves facilitate the absorption of supplied compounds. Indeed, the leaf shape and elevation angle allow for the fertilizers to accumulate and flow towards the leaf mid-rib and stem (Fernández et al., 2017). Maize (*Zea mays* L.) is an important cereal crop with a wider range of uses than other cereals (Olaniyan, 2015). It ranks 3rd after wheat and rice in the world's production of cereal crops and is known as the king of grain crops (Cooper et al., 2014). Maize is valuable as a source of food, feed, oil, and biofuel (Badr et al., 2020). Megagreen is an ecological foliar fertilizer, produced from natural mineral calcite by patented technology of tribomechanical micronisation and activation (TMA), a process in which nanoparticles are produced by micronisation and activation induced by friction dynamic process between the contact surfaces caused by great speed in very short time intervals. By chemical composition Megagreen is calcium fertilizer in combination with trace elements. The effects of this fertilizer are: increases the natural resistance of plant, incseases yield, shortens the time of ripening, increases plant resistance to diseases and attacks from pests, reduces the need for water in arid regions to 70% and prolonged time of storage of fruits (http://www.megagreen.com.mk/en/megagreen). The main component of this fertilizer is calcium, which is an essential element for plant growth and productivity. It plays a structural role in cell walls and membranes, counter-cation for inorganic and organic anions in the vacuole, acts as an intracellular messenger in the cytosol, and helps plants resist different environmental stresses (Hochmal et al., 2015, Kapilan et al., 2018). Naeem et al. (2018) found that foliar application of Ca (40 mg L^{-1}) is effective in improving maize growth and productivity. In addition, Naeem et al. (2017) suggested that Ca application is effective to make maize plants survive under drought conditions. The aim of this study was to determine the effect of foliar spraying with ecological fertilizer Mega-green on corn yield and grain quality.

MATERIAL AND METHODS

Test materials and experimental design

The test was carried out in the Skopje region, the north part of the country (x = 4655490.91; y = 7537392.11) at the fields of Institute of agriculture, Skopje. The field trial was carried out during the 2008-2009 period, on ***maize crop*** belonging to the ***hybrid ZP 677***, which is belongs to the group of late hybrid (FAO 600), with potentional yield above 13.5t/ha, with high quality (ZP 677 (mrizp.rs)).

Table 1. The main characteristics on ZP 677

Hybrid traits			
Agronomic properties		Quality parameters	
Germination	4	Hard fraction	59%
Growing	4	Soft fraction	41%
Stay green	5	Proteins	8.67%
Free moisture	3	Fats	4.55%
Plant height	255 (cm)	Starch	71.49%
Days to maturity	130-135	Yield of dry matter	17.90 t/ha
No. of grain rows	14-16	Cellulose	2.45%
Colour of corn cob	red	Coefficient of digestion	71.05
Length of corn cob	26-28cm		

*Scale from 0 to 5

Field trials have been organised according the method of randomized complete block design, with 4 (four) variants in 3 (three) replications on total 12 parcels (parcel area of 14.7m^2= 80 plants) for each year. Due to the application of the crop rotation measure, the trial in the second year of the research was carried out on the adjacent plot.

The Skopje valley is in the continental-submediteran climatic region, where the influence of submediteran climate is weaker than the continental climate (Filipovski et al. 1996). The growing seasons were favourable for corn growing (Table 2 and 3).

Laboratory analyses

According the latest classification of the soils in North Macedonia, the trial was carried on alluvium soil type, subtype calcaric (Filipovski, 2006). According to the results from the mechanical composition analysis and the Scheffer & Schachtschabel classification, the soil is classified as clay loam. The favorable mechanical composition allows these soils to have good physical properties that ensure high yields with the practice of modern agro-technicals maesures. This type of soil is characterized by good water permeability and relatively good water retention, which is of great importance for the proper supply of water to the plants during the vegetation.

Soil samples from two depth (0-20 and 20-40cm) were collected to determinate some properties. Standardized laboratory methods were used to test the basic chemical soil properties, as: pH value - 1:2.5 (v/v) suspension of soil in H_2O, with a glass electrode; free $CaCO_3$ by the volumetric method; content of organic matter by Tjurin – modification by Simakov; available form of nitrogen (N) by method of Bremner; available forms of phosphorus (P_2O_5) and potassium (K_2O) by the AL method according to Egner-Riehme; available form of iron (Fe), manganese (Mn) and cink (Zn) by the DTPA method (Jones, 2001) and mechanical composition by pipet method with Na-pirophosphate.

During the research period, standard commercial agro-technical practices were implemented. Soil tillage and soil fertilization with mineral fertilizers were performed each year in the spring with 400kg/ha NPK 15:15:15 and 300 kg/ha KAN 27%. During the vegetation in both research years, four irrigations were performed in furrows, starting from the 7-8 leaf stage. Treatments consisted three levels of the fertilizer with in a concentration of 0.3; 0.6 and 0.9 percent of fertilizer Megagreen and control variant (without fertilizing).

Megagreen is ecological foliar fertilizer, made of calcite, micronized by a new tribomechanical technology. Main components of fertilizer are: $CaCO_3$ - 82.3 %; SiO_2 - 5.56 %; MgO - 3.02 %; CaO -41.7%; Fe - 8783 mg/kg; Mn - 156 mg/kg; Se - 0.24 mg/kg.

The different concentrations of fertilizer were applied four times during the growing period with back sprayer and 4.8 L solution for each variant, starting from the stage of 7-8 leaves (V7) in an interval of 10-15 day.

Research variants of foliar treatments were as follows:
Variant 1 – control (without fertilizer)
Variant 2 – 0.3 % solution of Megagreen
Variant 3 - 0.6 % solution of Megagreen
Variant 4 - 0.9 % solution of Megagreen

Data collection and analysis

- ✓ Total corn grain yield - was determined from all plants of each replicate and was reduced to tons per hectare at 14% moisture;
- ✓ Corn grain analysis- during the harvests, the samples of corn grain were taken from each variant for some chemical analysis, like: total amount of Mn, Zn, Co and Fe by ISP-AES technique after their microwave digestion using $HNO_3 + H_2O_2$ (Cvetković, 2002).

Statistical analysis

Analysis of variance was performed using the SPSS 14.0 software (SPSS Inc., 2005) and correlation was calculated at 0.05level.

Table 2. Average monthly temperature (^oC)

Parameters	**Year**	**Month**												Average temperature during vegetation	Average yearly temperature
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
Average monthly temperature	2008	1.4	5.6	9.6	13.7	18	22.4	24.2	26	17.9	14.2	8.5	5.0	20.36	13.90
	2009	1.0	3.3	7.1	13.4	18	21	24.1	23.8	19.3	12.7	8.3	4.5	19.93	13.00
	91/05	0.7	2.4	7.5	12.2	17.6	22.3	24.5	24.2	18.9	13.2	6.3	1.2	19.95	13.48

Table 3. Average monthly rainfalls (mm)

Year	Month												Amount of yearly rain	Amount of rain in vegetation
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2008	7.7	0.5	29.8	18.7	40.7	46.9	57.8	24.8	78.5	27.3	37.5	68.3	438.5	267.4
2009	79.8	12.2	68.6	65.3	70.2	104.3	10.2	50.2	11.1	52.9	56.1	79.6	660.5	311.3
‘91/05	35.7	26.3	31.3	47.9	48.7	41.1	37.1	31.8	39.1	46.8	45.1	55.4	486.3	245.7

Table 4. Soil properties at test site

Year	Depth	$CaCO_3$	pH		Organic matter	Available					
			H_2O	KCl		N	P_2O_5	K_2O	Fe	Mn	Zn
	cm	%			%	mg/100g soil			ppm		
2008	0-20	13,97	8.0	7.0	1.58	11.2	26.38	16.51	9.58	34.40	2.62
	20-40	15,85	8.0	7.0	1.16	2.8	13.38	14.94	10.30	24.30	2.16
average	0-40	14.91	8.0	7.0	1.37	7.0	19.88	15.72	9.94	29.35	2.39
2009	0-20	12.74	8.0	7.2	1.85	10.8	40.56	54.04	12.00	17.90	10.3
	20-40	15.28	8.1	7.1	1.94	2.57	42.49	30.88	12.15	18.95	6.65
average	0-40	14.01	8.05	7.15	1.89	6.68	41.52	42.46	12.07	18.42	8.47

RESULTS AND DISCUSSION

Corn grain yield

Fertilizers can improve grain quality in maize by ensuring a consistent and targeted supply of essential nutrients, such as nitrogen, phosphorus, and potassium, as well as micronutrients, such as zinc and iron (Zhang et al., 2019). By improving nutrient use efficiency and reducing nutrient losses, these fertilizers can enhance the availability and uptake of nutrients by maize plants, leading to increased kernel size, weight and nutrient content (Wang et al., 2018).

Table 5, shows the yield results of 3 different concentrations of Megagreen depending on the year of production. The higher yield was noted at all treated variants compared with control variant, but without significant difference. The Variant 3 (in 2008) and the Variant 2 (in 2009) have the highest corn grain yield.

Corn grain is the primary product for which this crop is grown, except when it is cultivated for silage, in which case the entire plant is utilized. As a result, grain yield is the most critical factor, with all agronomic practices focused on maximizing yield per unit area. This directly influences the income generated from the crop. Yield is a fundamental indicator of production success, closely followed by considerations of both quality and quantity. Grain yield per unit area is primarily determined by the genetic potential of the variety, followed by soil, climatic, and agrotechnical conditions. One of the most significant factors in achieving high and high-quality yields is the proper and timely application of fertilizers.

The amount of fertilizer applied is based on realistic expectations regarding yield and the plant's nutrient requirements, taking into account the nutrients available in the soil (Davis and Westfall, 2009).

In all treated variants and in both experimental years, a higher yield was achieved compared to the control variant, although these differences were not statistically significant at the 0.05 or 0.01 probability levels. In the first experimental year, Variant 3 recorded the highest yield of 10488.33 kg/ha, while the control variant had the lowest yield of 9166 kg/ha. In the second experimental year, Variant 2 achieved the highest yield at 9919 kg/ha, whereas the control variant recorded 9836 kg/ha, the lowest yield.

When considering the average values over the study period (2008/09), Variant 3 had the highest average yield of 10178.66 kg/ha, while the control variant had the lowest average yield of 9501 kg/ha.

In a study of several maize hybrids with FAO ratings from 500-700 under irrigated conditions, including the hybrid ZP 677, an average grain yield of 8.8 t/ha was obtained for ZP 677 (Nikolovski et al., 1995).

Research conducted in 2006 on foliar feeding of maize with CoRon and High NRG-NR fertilizers during the V6 phase showed an increased yield in the treated variants compared to the control. However, no statistically significant differences were observed between the results (http://agroliquid.com).

The yield of corn is influenced by a wide range of factors, and the impact of these factors has been extensively studied by numerous researchers.

According to Saric et al. (1991), some studies suggest that an increased concentration of CO_2 in the air improves the uptake of nitrogen (N), phosphorus (P), and potassium (K) from the soil, which ultimately enhances the yield. The primary component of the fertilizer is calcium carbonate ($CaCO_3$), which, when absorbed by the plant leaves, rapidly decomposes into calcium oxide (CaO) and carbon dioxide (CO_2). This process ensures a constant supply of both carbon dioxide and calcium to the plant.

Despite differences in climatic conditions between the years, particularly in the distribution of precipitation during the growing season, and variations in soil fertility due to the experiment being conducted on two different plots, foliar feeding still had a positive but not significant effect on the corn grain yield in both experimental years compared with control variant.

Table 5. Grain yield at corn hybrid ZP 677 (t/ha)

Variant	Yield		
	2008	2009	2008/09
1	9.166	9836	9.501[a]
2	9.241	9.919	9.580[a]
3	10.488	9.869	10.178[a]
4	9.952	9.872	9.912[a]

*Different letters (a, b) indicate significant differences among treatments

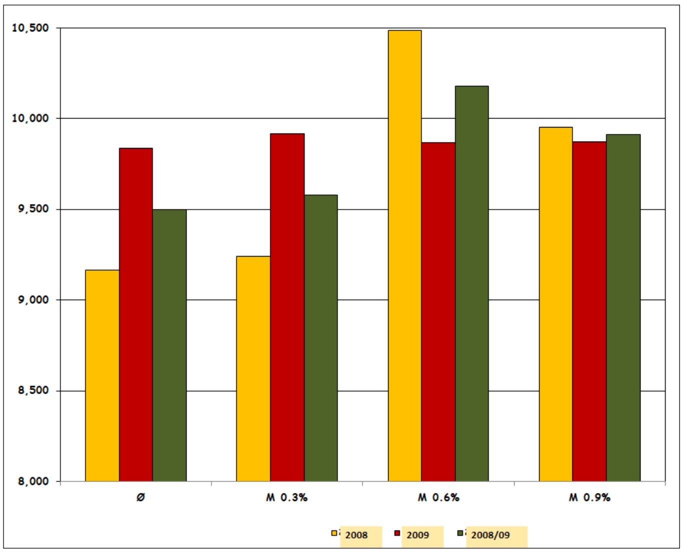

Figure 1. Grain yield at corn hybrid ZP 677 (t/ha)

Grain quality

The basic precondition for obtaining high quality corn yield is provision of sufficient available quantities of necessary during the whole vegetation. All elements indispensable for normal life cycle is named as, necessary elements. This group includes C, O, H, N, P, K, Ca, Mg, Fe, B, Mn, Zn, Mo and Co (Saric et al. 1991).

The discovery of trace elements' crucial role in plant life has helped explain a range of phenomena in plants that were previously attributed to diseases caused by microorganisms. These phenomena, which include certain anatomical and morphological changes, are now understood to result from deficiencies or imbalances in trace elements. Moreover, ongoing scientific advancements have uncovered many physiological and biochemical processes in which trace elements play an essential role.

The data on the trace element content in corn grains, as shown in Table 6, reveal interesting findings. Iron, in particular, is vital for plants due to its dual valency and its ability to form chelates. Through the cytochrome system, iron contributes to oxygen transfer by participating in the enzyme-driven processes of catalase and peroxidase, functioning as an oxidation-reduction agent.

Analysis of the corn grains revealed that the highest average iron content, 25.67 mg/kg, was found in the treated Variants 2 and 4. In comparison, the control variant had an average iron content of 25 mg/kg, while Variant 3 had the lowest, with 23.5 mg/kg. This makes Variant 3 the one with the least average iron content in the experiment.

Further analysis of Table 6, shows that the iron content in the first experimental year was significantly higher than in the second year. This difference can be attributed to the greater iron concentration in the leaf mass at the end of the first year's vegetation period.

Although differences in the iron content between the fertilizer-treated variants and the control variant are evident, these differences are not statistically significant at the 0.05 level.

According to Hongxing & Yu-kui (2010) the Fe content in corn grain is 28.89 mg/kg.

Manganese is a microelement whose concentration in plants primarily depends on the plant species, as well as the specific part or organ of the plant. Its role is similar to that of magnesium, as it can non-specifically replace magnesium in the activation of decarboxylase and dehydrogenase in the Krebs cycle. Manganese is also crucial for the reduction of nitrates, and its deficiency can lead to the accumulation of nitrates due to impaired reduction. When plants have an adequate supply of manganese, their need for nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) decreases without negatively impacting yields. Thus, manganese is important for the efficient use of other soil elements. Young plant organs tend to contain a higher concentration of manganese. The average manganese content in plants ranges from 50 to 250 ppm, with wheat grain containing around 34 ppm, barley 17 ppm, and corn grain only 6 ppm (Vukadinović and Loncarić, 1997).

According to the data presented in Table 6, all variants with different concentrations of foliar fertilizers exhibited a higher average manganese content compared to the control variant. The highest average manganese content, 6 mg/kg, was found in Variant 4, while the lowest average content, 5 mg/kg, was observed in the control variant.

From the results, it is evident that Variant 4 consistently showed the highest manganese content in corn grain over both experimental years. Although all treated variants demonstrated an increase in manganese content, suggesting that fertilization had a positive effect on the manganese levels, only Variant 4 showed a statistically significant difference compared to the control variant.

In their study on the determination of trace elements to assess the pollution and safety of corn grown in Beijing, China, Hongxing and Yu-kui (2010) found that the manganese content in corn kernels in that region was 4.40 mg/kg.

Zinc, as a microelement, plays a critical and wide-ranging physiological role in plants. It influences the metabolism of various substances, particularly proteins, and is involved in the absorption and transport of phosphorus. Zinc also enhances the plant's resistance to diseases, drought (by reducing transpiration), and low temperatures. Among the microelements, zinc has the most significant impact on the growth of corn. Chronic zinc deficiency is well-documented and has been shown to adversely affect the health of both humans and animals.

In the case of corn grain, the treated variants (3 and 4) exhibited higher zinc content compared to the control variant. The control variant had an average zinc content of 21.17 mg/kg, while Variant 2 showed a slightly lower average content of 20.67 mg/kg. Variant 4, however, had the highest average zinc content in the grain, with 24.17 mg/kg.

The increased zinc content in the grain of the treated variants suggests that foliar fertilization positively affected the zinc concentration. However, only the difference between Variant 4 and the control variant was statistically significant at the 0.05 probability level. According to Bensony (cited by Jevtić, 1977), the average zinc content in corn grain at 15% moisture is 17 mg/kg. Additionally, research by Djerija and Matica (1976, cited by Kastori, 1983) indicates that, depending on the treatment, zinc content in the germ of corn grain ranges from 147 to 169 ppm, while in the endosperm, it ranges from 5 to 7 ppm. The significant accumulation of zinc in the tuber highlights its crucial role in seed germination processes.

Table 6. Content of microelements in corn grain

Var.	Fe			Mn			Zn		
	2008	2009	2008/09	2008	2009	2008/09	2008	2009	2008/09
1	26.0000	24.0000	25.0000^{a}	4	6	5.0^{b}	21.6667	20.6667	21.1667^{b}
2	31.3333	20.0000	25.6667^{a}	6	5	5.5^{ab}	24.6667	16.6667	20.6667^{b}
3	25.3333	21.6667	23.5000^{a}	6	5	5.5^{ab}	24.0000	20.0000	22.0000^{ab}
4	28.6667	22.6667	25.6667^{a}	6	6	6.0^{a}	26.3333	22.0000	24.1667^{a}

*Different letters (a, b) indicate significant differences among treatments

CONCLUSION

Despite differences in climatic conditions between the years, particularly in the distribution of precipitation during the growing season, and variations in soil fertility due to the experiment being conducted on two different plots, foliar feeding still had a positive but not significant effect on the corn grain yield in both experimental years compared with control variant. When considering the average values over the study period (2008/09), Variant 3 had the highest average yield of 10,178.66 kg/ha, while the control variant had the lowest average yield of 9,501 kg/ha.

The statistical analysis showed significant positive effect of foliar application in concentration of 0.9% on the content of Mn (5-6mg/kg) and Zn (22-26.33 mg/kg) in corn grain. The results revealed that foliar spray of Ca did not significantly affect the concentration of Fe (23.5-25.66 mg/kg) in the corn grain.

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INFLUENCE OF SOME MYCORRHIZAL PRODUCTS ON VINE PLANTING MATERIAL PRODUCTION

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ABSTRACT

During the period 2018 - 2020, a field trial was carried out to establish the influence of some mycorrhizal products (Rhizo vam basic and Dynocarb MYC) on the quantity and quality of standard vine planting material obtained from Cabernet Sauvignon variety grafted to Berlandieri X Riparia SO4 rootstock. Rhizo vam basic was applied in two ways - by mixing the particles with the soil during the bed formation (dose 0.2 l/m2) and introducing the product directly to the root formation area (dose 0.005l per cutting). Dynocarb MYC was applied through the irrigation water by means of the drip irrigation system. The variants were set in beds without mulching the surface with polyethylene film. The dynamics of shoot growth was monitored and the ratio of standard rooted vines was reported. An analysis was made of the biometric indicators characterizing their habitus. The application of the mycorrhizal product Rhizo vam basic resulted in increase in the yield and quality of the vine planting material as the effect was more significant when the carrier clay particles were evenly mixed in the cultivated soil layer during the bed formation. Dynocarb MYC stimulated shoot growth and the formation of mature growth with greater length and mass, but no significant increase in the ratio of standard rooted vines was found after triple treatment with the product.

Key words: *Vine, planting material, mycorrhiza, growth.*

INTRODUCTION

The application of biological products based on fungal cultures to increase the quantity and quality of yields from various agricultural crops, as well as to enhance the decorative value of ornamental plants, has been a way to reduce pollution with chemical agents. Mycorrhiza and the choice of fertilization have affected the growth rate, being a valuable factor in minimizing the cost of nutrition (Kubiak, 2006, Yablonskaya and Knishkaite, 2014). The beneficial effects of arbuscular mycorrhizal (AM) fungi on plant productivity and soil health have been essential for the sustainable management of agroecosystems (Gianinazzi et al., 2010). The analysis of the results from the application of arbuscular mycorrhiza has shown the prospects of this method in viticulture. It has represented a direct link between the soil and the vine and the study of its impact would allow assessing its contribution to the "terroir", which has been undoubtedly of particular significance in viticulture (Trouvelot et al., 2015).

This method enabled the development of a symbiosis between fungi and vines, where the fungal hyphae took over the function of root hairs, and the vine provided the fungus with the necessary substances obtained during photosynthesis. That was based on a biotrophic exchange of nutrients between the plant and the fungal partner: the host plant (in this case, the vine) supplied the biotrophic partner with carbon (C), while it improved the plant's ability to uptake water and nutrients from the soil (Smith and Read, 2008).

It had been found that the application of AMF optimized the growth processes and rhizogenesis of grafted vine cuttings (Kara, 2011). There were differences in the ability to form mycorrhiza between vine rootstocks; however, there were other factors that much more significantly influenced the colonization of roots by AM fungi. Rootstocks that conferred greater vitality, especially Ruggeri 140, Kober 5BB and SO-4, had consistently higher levels of root colonization (Schreiner, 2023.) Controlled mycorrhization might significantly improve the growth dynamics of vine plants (Tsvetkov et al., 2017). (Aguín et al., 2004). Inoculation of arbuscular mycorrhizal fungi in rooting media could be a useful tool to improve productivity in vine nurseries (Aguín et al., 2004). Such inoculation had been found to increase mycorrhizal colonization and root biomass formed by young vines (Cheng and Baumgartner, 2004).

Further to the positive impact on the biological productivity of grafted grapevine cuttings, the application of arbuscular mycorrhizal fungi also led to an increase in their adaptive potential (Yurkov et al., 2013). Symbiosis with fungi of the genus Glomus induced tolerance of grapevine plants to water deficit and soil salinization (Nikolaou et al., 2003; Schreiner and Linderman, 2005; Belew et al. 2010; Khalil, 2013). It had been established that the application of arbuscular mycorrhizal fungi in grapevine nurseries reduced the infection with other fungal species that might show pathogenic effects (Nogales, 2009).

The objective of the present study was to investigate the response of grafted grapevine cuttings during their rooting after controlled colonization by different products with arbuscular mycorrhizal fungi and to define an appropriate manner to introduce these products into the soil.

MATERIAL AND METHODS

During the period 2018 – 2020, a field experiment was carried out in the nursery within the territory of the Experimental Base of the IVE, Pleven (43.42°N 24.62°E and 140 m altitude). The soil type of the area where the vineyard was located was leached chernozem, formed on clay loess. In mechanical composition, it was heavy sandy loam, with good water-physical properties (Krastanov and Dilkova, 1963).

Cuttings of the Cabernet Sauvignon variety were used, grafted on the Berlandieri x Riparia SO4 rootstock and planted in two-row beds without mulching with polyethylene film. The planting depth was 15 cm with inter-row distances of 7-8 cm between the cuttings and 50 cm between the rows in the bed. The grafted cuttings got water through the drip irrigation system with one irrigation wing per bed, located between the two rows of vines (Tsvetanov, 2019).

Mycorrhiza was applied by introducing two products:

1. Rhizo vam basic – containing propagation material of natural, non-genetically modified arbuscular mycorrhizal fungus (*Glomus intraradice*), fixed on expanded clay particles with a diameter of 2 to 4 mm, in an amount of 100,000 infecting units per litre. It was applied once in the root formation soil layer.

2. Dynocarb MYC – a water-soluble multicomponent product for soil application, containing propagation material of the mycorrhizal fungus Rhizoglomus - (*Glomus intraradice*) in high concentration. The product was rich in calcium, magnesium, silica, iron, zinc, seaweed extract (algae), and beneficial mycorrhizal hyperbacteria such as Panibacillus, Bacillus and Pseudomonas. It combined the products Dynocarb and the mycorrhizal vaccine Rhizovam basic. It was introduced three times – every two weeks after the thirtieth day from the planting of the cuttings into the nursery.

The study variants were as follows:

V1 – application of Rhizo vam basic by mixing the particles with the soil when forming the bed (dose 0.2 l/m^2);

V2 – application of Rhizo vam basic directly into the rooting area (dose 0.005l per cutting);

V3 – application of Dynocarb MYC with the irrigation water (dose 0.05 kg/da);

K - control without mycorrhiza

The experiment was set up in four repetitions of 50 cuttings by the long plot method.

The following indicators were monitored:

- shoot growth in dynamics (cm) – the recordings were made every 10 days until growth ceased. The first recording was made thirty days after the cuttings were planted in the nursery.
- yield of standard rooted vines (%);
- length and fresh mass of the mature part (mm);
- diameter of the second internode from the base of the main shoot (mm)
- number of developed roots (< and > 2mm)

The data were processed by analysis of variance (Dimova and Marinkov, 1991).

RESULTS AND DISCUSSION

The shoot growth dynamics of the grafted cuttings showed a strong influence of the mycorrhizal products (Figure 1). Throughout the entire period of the study, the most intensive growth was observed in the variant in which Rhizo vam basic was introduced by mixing the particles with the soil during the formation of the bed (V1), followed by Dynocarb MYC with the irrigation water (V5). The data for V1 revealed the greatest growth intensity throughout the entire vegetation period, due to the uniform saturation of the soil layer with mycorrhizal particles already during the formation of the bed. That allowed the creation of symbiosis with *Glomus intraradice* from all offshoots of the root system. In V5, the greatest growth intensity was recorded after the third application of the product with the irrigation water, when the fungal organisms reached a sufficiently high concentration in the soil to colonize the already formed root system of the grafted cuttings. At the end of the growing season, the greatest shoot length – 867.7 mm (V1) and 755.0 mm (V5), was recorded in these two variants, respectively. All variants with mycorrhiza had stronger growth compared to the control, but at the end of the growing season, a significant difference in the shoot length was recorded only in V1 (at p = 1%).

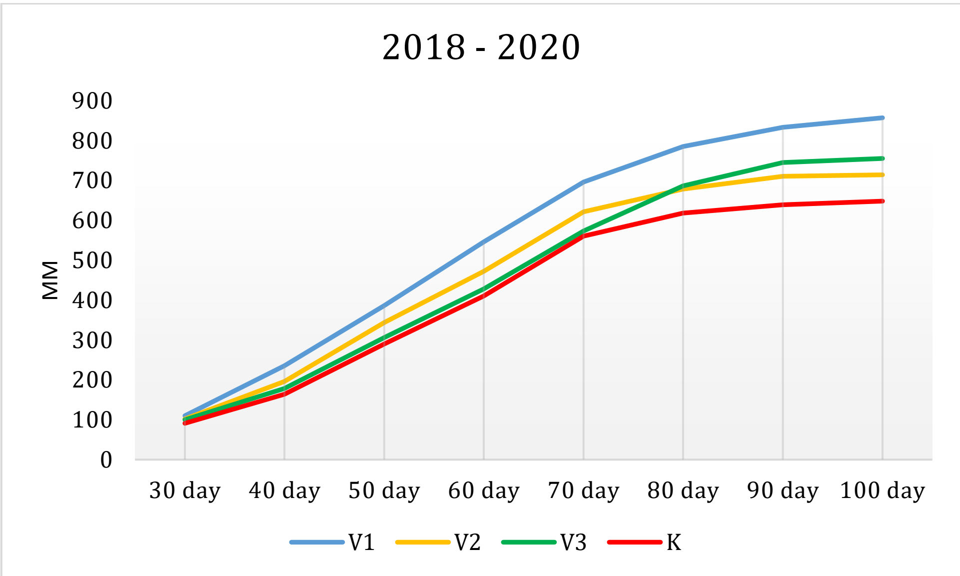

Figure 1. Main shoot growth in dynamics on the average for the period 2018–2020

The variant with the most intensive growth (V1) also provided the highest yield of standard rooted vines (table 1). This trend remained stable and significant throughout all years of the study. The variant with Dynocarb MYC (V5) was second, despite the variation over the years. The introduction of Rhizo vam basic into the bed (directly in the root formation area) did not lead to a significant increase in the yield of standard vines – the poor mobility of *Glomus intraradice* did not allow its uniform distribution and reduced the efficiency of the symbiosis. The difference in the yield of standard vines on the average for the period of the study between V1 (Rhizo vam basic, introduced mixed in the bed) and the control was significant (p=5%), as was the difference between V1 and the rest variants.

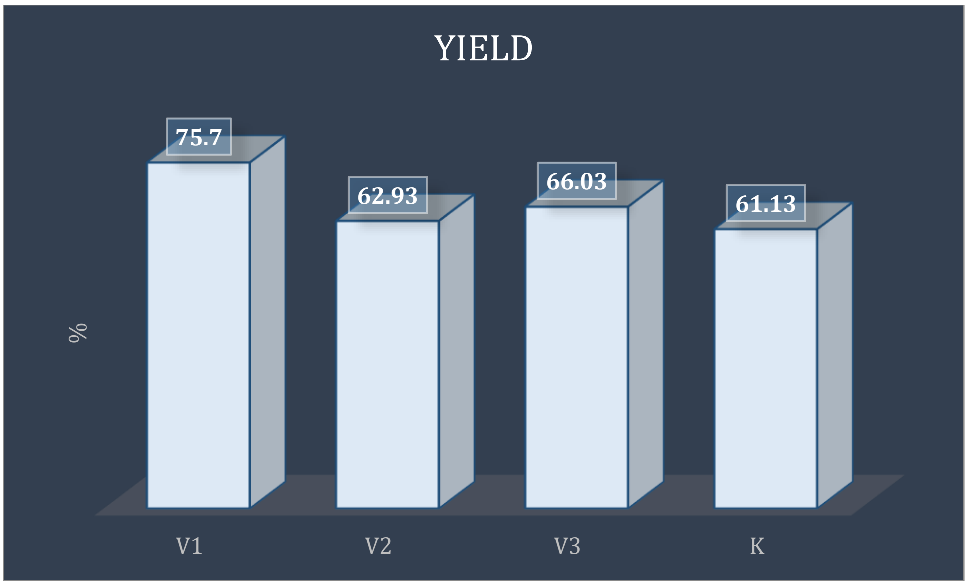

Figure 2. Yield of standard rooted vines during the period 2018 – 2020

To characterize the quality of the planting material, biometric measurements were made of the obtained grafted rooted vines (Table 1). In the variants with mycorrhiza, on the average for the period, the length and mass of the mature growth were greater than those in the control. The vines from the variant with mixed application of the mycorrhizal product Rhizo vam basic had the highest values of the indicator. The differences compared to the control and the other variants were of good probability (p = 1%). In the vines of this variant, the largest diameter of the main shoot at the second internode was also measured (p = 5%). In the other variants, it did not differ significantly from that in the controls. The vines from the variants treated with the mycorrhizal products and the control developed a root system with approximately the same total number of roots. Differences were observed only in the formation of roots with a diameter of over 2 mm (skeletal). The application of Rhizo vam basic mixed in the bed resulted in a significant increase in their number (p = 5%).

Table 1. Biometric characteristics of grafted rooted vines on the average for the period 2018 – 2020

Variant (V)	Mature growth length (mm)	Mature growth mass (g)	Number of roots			Diameter of second internode (mm)
			d>2 mm	d<2 mm	Total number	
V1	1326.0**	29.37**	5.5*	9.6 ns	15.1 ns	6.8*
V2	943.0 ns	19.50ns	4.3 ns	10.6 ns	14.9 ns	5.7ns
V3	1076.0 ns	23.00ns	4.7 ns	11.2 ns	15.9 ns	6.3ns
K	821.3	16.37	3.8	10.1	13.9	5.5

Significant at p = 5% (*); p = 1% (**); p = 0.1% (***); p < 5% (ns)

CONCLUSIONS

The application of the mycorrhizal product Rhizo vam basic led to an increase in the yield and quality of the vine planting material, the effect being more significant when the carrier clay particles were evenly mixed into the arable soil layer when forming the bed.

The application of Dynocarb MYC stimulated the growth of shoots and the formation of mature growth with greater length and mass, however after three treatments it did not cause a significant increase in the yield of standard rooted vines.

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STUDY OF THE INFLUENCE OF GREEN PRUNING OPERATIONS ON THE RATE OF MATURATION AND STRENGTH OF GROWTH OF SUMMER PLANTS IN THE VARIETY MUSKET KAILASHKI

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ABSTRACT

The study was conducted at the Experimental Base of the Institute of Viticulture and Enology - Pleven during the period 2017 - 2020. The influence of some basic green pruning operations on the degree of ripening and the strength of growth of the shoots of the Misket kailashki variety was studied. It was established that the vines without applied green pruning operations and those with thinning of the clusters and lightening in the zone of the clusters are characterized by a stronger growth, as in all reported indicators - average length of one shoot, average length and thickness of one internode and mass of a one-year mature growth, the differences compared to the variants with performed crushing are mathematically proven. On the other hand, following the same trend, the growth strength of the vines in all variants was significantly greater during the wetter and cooler years 2017 and 2020 and significantly less during the medium-dry and very hot vegetation of 2019. In terms of length of the mature part of the shoot and the percentage of the mature part in relation to the total length of the shoot, determining the degree of maturation of the shoots, the opposite trend is observed in the values of the reported indicators. Pruning, like green pruning, contributes to better shoot maturation, with the highest values measured in the dry and hot climate of 2019.

Key words: *vine, green cuttings, degree of ripening, growth vigor, one-year mature growth.*

INTRODUCTION

Shoot maturation had been of practical significance. It was associated with the cold resistance of buds and shoots, as well as the quality of the cuttings and rootstocks in the production of planting material (Braykov et al., 2005). The level of vine shoot maturation had been in direct correlation with the genetic determination and biological specifics of the variety, the weather conditions during the growing season and the applied agricultural practices. This indicator provided an accurate idea of the completion of shoot growth and the stage of complex biochemical processes occurring in the grapevine, determining the subsequent stages of hardening of the vines and the levels of their cold resistance (Simeonov, 2017).

The vigour of vines had been a basic biological feature of the variety and indicated their growth and fruiting potential, which might be objectively determined by the degree of development of the root system, shoots, old wood, as well as by the reserve nutrients (Negrul, 1959). It could be measured in length (m), mass (kg) or visually, assessing the variety potential to form many or few, strong or weak lateral shoots (Katerov et al., 1990). The individual shoot growth was directly dependent on the number of shoots left. It was weaker when more shoots were left, but when their number on the vine was reduced below a certain minimum limit, although the individual growth of the shoot was enhanced, the vigour of the vine weakened because of the decrease in the total size and leaf mass (Nikov and Rangelov, 1991).

Green pruning treatments had different effect on the vegetative parameters, determining the growth strength and the degree of maturation of the shoots. The study of the physiological effect and the after-effects of green pruning was also of interest with a view of a more complete understanding of the biological phenomena of self-regulation and plasticity of grapevine (Braykov et al., 2005).

The annual mature growth was well correlated with the leaf area and therefore could be used as a practical indicator of the presence of assimilates (Smart et al., 1985). During shoot topping the annual growth and leaf number of the main shoots dropped down, but leaf number of the lateral shoots changed in the opposite direction and went up significantly (Slavcheva et al., 2006). An increase in the yield/growth ratio, the economic efficiency coefficient and the leaf productivity was recorded. The growth mass could increase with a decrease in the number of clusters per vine, even at relatively low loading (Bravdo et al., 1984, 1985a; Fisher et al., 1977; Kliwer and Weaver, 1971; Kliwer et al., 1983; Reynolds et al., 1986, 1994a, 1994b, 1994c; Weaver and McCune, 1960). According to some authors, the growth mass was not affected by the change in the number of shoots per vine (Freeman et al., 1979; Reynolds and Wardle, 1989; Reynolds et al., 1994 b, 1994 c), which indicated that vine could enhance the vitality of individual shoots to compensate for a decrease in the number of shoots during suckering (Naor et al., 2002). Regardless of the type of summer hedging, all green pruning treatments should be applied appropriately and at the right time. Improper and untimely shoot topping and pinching off might cause strong development of lateral growth points (Merzhanian, 1953). That led to the uptake of valuable nutrients from a large number of newly developed shoots, which slowed down the shoots growth, poor wood maturation, decrease in the sugar content in grapes, and the buds and shoots became less cold resistant.

The objective of this study was to investigate the impact of the green pruning treatments – cluster thinning, shoot topping at the end of June and at the end of July, and thinning in the cluster zone on the degree of maturation and the growth vigour of the shoots of the interspecific white wine variety Muscat Kailashki.

MATERIAL AND METHODS

The object of the study was the interspecific white wine variety Muscat Kailashki. It was selected through interspecific hybridization of the varieties Muscat Hamburgski x Villard Blanc 12 375 in IVE-Pleven. The variety was distinguished by very good fertility, practical resistance to mildew and showed enhanced resistance to grey rot and low winter temperatures. Muscat Kailashki was a very late-ripening variety, suitable for the production of original white dry and dessert wines, as well as for wine distillate. (Roychev, 2012).

The experimental plantations were located in the Tomovskoto area, in the region of the Experimental Base of the Institute of Viticulture and Enology – Pleven. The planting distance was 2.50 x 1.30 m, and the vines were grown on semi-high training (double-sided cordon) with a stem height of 1 m. The experimental vines were short-pruned, on spurs, with a loading of 18 eyes (9x2). The total number of the experimental vines per variant was 20 (4 repetitions x 5 vines). The age of the plantation of the studied variety during the research period was 15 – 20 years.

The shoot topping was performed manually, simulating contour mechanized pruning. In all variants, suckering was done at the base and along the stems of the vines. After counting the inflorescences, the vines were distributed into five variants with approximately the same average number of inflorescences. For the studied variety, a curve of the distribution of inflorescences was made each year as the vines with the least and most inflorescences were not included in the variants (Belberova, 2023).

The study consisted of the following trial variants:

V_1 – control – no summer pruning

V_2 – shoot topping – June;

V_3 – rationing (thinning) of the clusters – June – 1/3 (30 – 35%) of the clusters were removed in total for the variant;

V_4 – shoot topping – July;

V_5 – thinning in the cluster zone – it was carried out manually in the phase of veraison, as the leaves at the base of the shoots next to the clusters were removed.

To determine the growth strength of the vines, the shoots' maturation and the mass of the annual mature growth were reported. The degree of the shoots' maturation was recorded visually in late October and early November. Each variant consisted of 20 shoots. After their maturation the following indicators were determined: average length per shoot (cm) – total mature and green part; average length per internode (cm) – 5th internode; average length of the mature part of the shoot (cm); ratio of the mature part compared to the total length of the shoot; average thickness of the shoot in the area of the 5th internode (mm). The annual mature growth mass (kg) was measured during pruning by weighing the one-year shoots of 20 vines from each variant (5 vines per repetition) (Katerov et al., 1990).

The statistical data processing was done by Fisher's analysis of variance at confidence levels of the differences (Student's criteria) p = 5.0 %, p = 1.0 %, p = 0.1 % (Dimova and Marinkov, 1999).

The climatic characteristics of the region were determined from data obtained from the hydrometeorological station in the town of Pleven and own meteorological cell located in the area of the trial.

RESULTS AND DISCUSSION

The analysis of the weather indicators in the area of the trial plantations showed a tendency of increasing the total temperature sum during the year and the vegetation season. The higher values of the recorded temperature rates in recent years, had confirmed the significant warming of the climate in Central Northern Bulgaria. The average monthly temperature was increasing, thus a rise in the average daily temperature was observed in the separate years by 0.7°C to 2.0°C.

In some of the years, a significant amount of precipitation was recorded, however it was distributed very unevenly per months and days. In recent years, it was noticed an increase in the absolute maximum and minimum temperature rates, compared to previous periods, as well as significant drought in the second half of the vegetation period, which affected the nature of these years, defining them as hot. According to the sum of rainfall and the soil structure, the average amount of precipitation was sufficient for the full moisture supply of the vine, but in certain years and months, that did not happen, due to the uneven distribution of the rainfall during the individual periods of the growing season. There was also a permanent trend towards a decrease in the average number of days with recorded rainfall during the vegetation period. Very light and/or intense rainfall often prevailed during the growing season, in which the water evaporated quickly and was not available to the root system of the vine or was lost in the form of surface runoff. That uneven distribution of precipitation was the cause of frequent and sometimes prolonged periods of drought.

The total sum of precipitation in 2017 was 947.8 mm/m^2, and during the growing season – 683.6 mm/m^2 (72.1%), with a coverage of 6%, which defined the period as very wet. The average daily temperature was 19.8ºC with a coverage of 33%, characterizing the period as hot, and the maximum was 27.9ºC with a coverage of 32%, which defined the period as very hot (Table 1).

The total sum of precipitation in 2019 was 654.3 mm/m^2, and during the growing season – 451.4 mm/m^2 (69.0%), with a coverage of 52%, which identified the period as moderately dry. Less precipitation that year had increased the average daily and maximum temperatures: the average daily temperature was 20.5°C with 19% coverage, which defined the year as very hot in terms of average daily temperatures; the average maximum temperature was 27.6°C with 13% coverage, which outlined the growing season as very hot also in terms of maximum temperatures (Table 1).

According to its climatic characteristics, apart from 2019, 2020 was also one of the warmest in the last 30-40 years.

Table 1. Climatic characteristics of the years from 2017 to 2020 according to the sum of precipitation and average average daily and maximum air temperature during the growing season

Year	Precipitation			Temperature					
				Average daily			Maximum		
	Amount	Probability	Character on year	Average daily	Probability	Character on year	Average daily	Probability	Character on year
	mm	%	-	°C	%	-	°C	%	-
2017	683,6	6,0	Moderately humid	19,8	33,0	Hot	27,9	32,0	Very hot
2019	451,4	52,0	Moderately dry	20,5	19,0	Very hot	27,6	13,0	Very hot
2020	452,8	42,0	Moderately humid	19,1	77,0	Cool	27,3	19,0	Very hot

The total amount of precipitation in 2020 was 672.3 mm/m^2, while during the growing season – 452.8 mm/m^2 (67.3%), with a coverage of 42%, which characterized the period as moderately wet. The average daily temperature was 19.1°C with a coverage of 77%, which determined the year as cool in terms of average daily temperatures, but the average maximum temperature was 27.3°C with a coverage of 19%, which defined the growing season as very hot in terms of maximum temperatures (Table 1).

Table 2 presents the indicators showing the degree of the shoots' maturation of the Muscat Kailashki variety.

In 2017, the average length of the mature part of the cane was the highest in the variant with shoot topping in July (V_4) – 113.40 cm and the smallest in the variant with shoot topping in June (V_2) – 96.75 cm. The shortest internodes were found in the variant with cluster thinning – V_3 (7.35 cm), and the longest in the control variant (V_1) – 7.88 cm. The control variant (V_1) had the highest average length per shoot (133.60 cm), and the variant with shoot topping in June – V_2 (111.10 cm) had the smallest one. Compared to the total shoot length, the ratio of the mature part was the lowest in the control – V_1 (64.71 %). The ratio of the ripe part in variant V_4 was (95.99%). The average thickness of the canes in the area of the 5th internode varied from 7.19 mm in the variant with shoot topping in July (V_4) to 7.53 mm in the variant with thinning in the cluster zone (V_5). The average thickness in the area of the 10th internode for the Muscat Kailashki variety in 2017 was within the range from 5.56 mm (V_1) to 5.68 mm (V_4).

In 2019, the average length of the mature part of the cane was the smallest in the variant with shoot topping in June (V_2) – 98.75 cm and the highest in the variant with shoot topping in July (V_4) – 119.05 cm. Data for the indicator of the average length at the 5th internode demonstrated that the shortest internodes were in variant V_3 (6.72 cm), and the longest in the control variant (V_1) – 7.35 cm. The variant with cluster thinning – V_3 (133.60 cm) had the greatest average length per shoot, followed by the variant with thinning in the cluster zone (V_5) – 126.80 cm, and the variant with shoot topping in June – V_2 (111.10 cm) had the smallest one. The differences between these variants were significant. The ratio of the mature part towards the total shoot length was the lowest in the variant with thinning in the cluster zone V_5 (64.11%). The highest ratio of the mature part was in the variant with shoot topping in June – V_2 (97.76%), followed by the variant with shoot topping in July – V_4 (96.74%). It was mathematically proven that variants V_2 and V_4 had a higher ratio of mature part compared to all other variants. The average thickness of the canes in the area of the 5th internode varied from 7.17 mm in the variant with shoot topping in June (V_2) to 7.73 mm in the variant with thinning in the cluster zone (V_5). The average thickness in the area of the 10th internode ranged from 5.64 mm (V_2) to 6.30 mm (V_1).

In 2020, the control (V_1) had the smallest average length of the mature part of the cane – 85.75 cm and the highest in the variant with thinning in the cluster zone (V_5) – 95.00 cm. The data for the indicator of the average length at the 5th internode showed that the shortest internodes were in the variant with thinning in the cluster zone – V_5 (6.52 cm), and the longest in the variant (V_4) – 7.13 cm. The variant with cluster thinning (V_3) had the greatest average length per shoot – 157.95 cm, followed by the control (V_1) – 151.90 cm, and the smallest in the variant with shoot topping in June – V_2 (112.15 cm). Compared to the total shoot length, the ratio of the mature part was the lowest in the control variant V_1 (64.00%). The highest ratio of the mature part was in the variant with shoot topping in June – V_2 (86.70%), followed by the variant with shoot topping in July – V_4 (85.41%). In the relatively wetter years of 2017 and 2020, the ratio of the mature part compared to the total shoot length was the lowest in the control variant V_1, which indicated that all green pruning treatments had a positive effect on the maturation of the shoots compared to the control in the separate years. The average thickness of the canes in the 5th internode zone ranged from 6.52 mm in the variant with thinning in the cluster zone (V_5) to 7.13 mm in the variant with shoot topping in July (V_4). The average thickness in the 10th internode zone varied from 4.95 mm (V_2) to 5.57 mm (V_1).

Table 2. Ripening rate and shoot growth strength of Muscat Kailashki variety variants for the period 2017-2020.

Variants	**Year**	**Average length of one shoot, cm**	**Average length of one internode, cm**	**Average length of the mature part of the shoot, cm**	**Ripe part relative to the total length of the shoot, %**	**Average thickness of rods in the zone of the 5th internode, mm**	**Average thickness of the shoot in the zone of 10th internode, mm**
V1 **Control**	2017	133,60	7,88	99,15	64,71	7,40	5,56
	2019	120,15	7,35	106,05	95,97	7,26	6,30
	2020	151,90	8,05	85,75	64,00	7,00	5,57
	Average	***135,22***	***7,76***	***96,98***	***74,89***	***7,22***	***5,81***
V2	2017	111,10	7,40	96,75	82,04	7,22	5,63
	2019	102,75	6,88	98,75	97,76	7,17	5,64
	2020	112,15	7,51	92,40	86,70	6,95	4,95
	Average	**108,67 +++**	**7,26 +++**	**95,97 n.s**	**88,83 +**	**7,11 +**	**5,41 ++**
V3	2017	128,15	7,35	96,80	73,11	7,50	5,59
	2019	133,60	6,72	99,75	94,55	7,32	6,22
	2020	157,95	9,24	91,20	70,65	7,04	5,38
	Average	**139,90 n.s**	**7,77 n.s**	**96,25 n.s**	**79,44 n.s**	**7,29 n.s**	**5,73 n.s**
V4	2017	117,40	7,48	113,40	95,99	7,19	5,68
	2019	115,30	7,27	119,05	96,74	7,21	5,93
	2020	139,80	7,78	93,00	85,41	7,13	5,11
	Average	**124,17 ++**	**7,51 ++**	**108,48 +**	**92,72 ++**	**7,18 +**	**5,57 +**
V5	2017	127,95	7,60	99,05	78,58	7,53	5,60
	2019	126,80	6,88	102,20	64,11	7,73	5,97
	2020	145,70	8,44	95,00	84,94	6,52	5,49
	Average	**133,48 n.s**	**7,64 n.s.**	**98,75 n.s**	**75,88 n.s**	**7,26 n.s**	**5,69 n.s**

GD(5,0%)=t*S~d=1,113; GD(1,0%)=t*S~d=1,677; GD(0,1%)=t*S~d=2,755

All experimental variants with applied shoot topping had a smaller average length per shoot compared to the control variant (V_1) – 135.22 cm and the variants with cluster thinning

(V_3) – 139.90 cm and with thinning in the cluster zone (V_5) – 133.48 cm, as the lowest values were obtained in the variant with shoot topping in June (V_2) – 108.67 cm, followed by the variant with shoot topping in July (V_4) – 124.17 cm. The results were also similar for the indicator average length per internode, where the average length at the fifth internode varied from 7.26 cm (V_2) to 7.77 cm (V_3). The mathematical analysis of these data revealed that the differences in the indicators of the length per shoot and per internode between the control and the variants with shoot topping in June and July were of very good probability and good probability, respectively.

The average length of the shoot mature part varied per years and was in direct correlation with the climate and the green pruning treatments. In years with drier and hotter growing season (2019), the rates of this indicator were the highest, while in cool and wet years (2020) – the lowest, which was due to the slower and weaker maturation of the shoots under such weather conditions. According to this indicator, 2017 occupied an intermediate position. The average length of the mature part of the cane for the three-year period was the highest in the variant with shoot topping in July (V_4) – 108.48 cm, followed by the variant with thinning in the cluster zone (V_5) – 98.75 cm and the variant with cluster thinning (V_3) – 96.25 cm, and the lowest rates had the variant with shoot topping in June (V_2) – 95.97 cm and the control (V_1), as the differences between the latter four variants varied within very narrow limits. The analysis of variance proved that the differences between the variant with shoot topping in July (V_4) and the rest of the variants and the control were significant.

The values of the indicator ratio of the mature part towards the shoot total length, on the average for the period, were the highest in the variants with shoot topping – in July (V_4) and in June (V_2). In the variant with cluster thinning (V_3), this ratio had an intermediate value. The smallest ratio of ripe part was recorded in the shoots of the control variant, without applied green pruning treatments (V_1). The differences between the variants with shoot topping in July and the control were of good probability while between the variant with shoot topping in June and the control – significant.

The average thickness of the canes in the 5th internode zone varied from 7.11 mm (V_1) to 7.29 mm (V_3), with the values of the indicator varying widely per years. In years with a wetter and cooler weather and stronger vegetative growth, the thickness of the 5th internode was smaller in all variants, as the differences between the control and the variants with shoot topping in June and July were significant.

The results obtained for the indicator average thickness of the shoots in the 10th internode zone were identical, where the rates were within the range from 5.41 mm (V_2) to 5.81 mm (V_1). For this indicator also, in years with a wetter and cooler climate and stronger vegetative growth, the thickness of the 10th internode was smaller in all variants, with the differences between the control and the variants with shoot topping in June and July were respectively of good probability and significance.

The values for the annual growth during the study period 2017 – 2020 are presented in Table 3. The variant with cluster thinning (V_3) had the highest growth mass – 0.89 kg. The lowest values were recorded in the variant with shoot topping in June (V_2) – 0.72 kg, which was a result of the nature of the treatments performed in this variant. In this variant, a large number of lateral shoots were formed, most of which did not ripen and that led to the lower rates of this indicator. The same, but to a lesser extent, could be said about the variant with shoot topping in July (V_4). From the statistical analysis of the data of this indicator, it was found that the variants without green pruning (V_1) and those without treatments of the green shoots – cluster thinning (V_3) and defoliation in the cluster zone (V_5) had significantly greater mass of the annual mature growth, compared to the variants with shoot topping carried out with different terms – V_2 and V_4.

Table 3. Mass of annual mature growth (kg) of Muscat Kailashki variety for the period 2017-2020

Variants Year	V_1 – Control	V_2	V_3	V_4	V_5
2017	0,87	0,70	0,97	0,80	0,86
2019	0,92	0,77	1,08	0,81	0,92
2020	0,82	0,68	0,61	0,75	0,80
Average	***0,87***	***0,72 +***	***0,89 n.s***	***0,79 +***	***0,86 n.s***

GD (5,0 %) = t*S~d=1,151; GD (1,0 %) = t*S~d=1,727; GD (0,1 %) = t*S~d=2,762

CONCLUSIONS

At the end of the growing season, the indicators determining the degree of maturation and the growth strength of the shoots, in all studied variants, had reached different parameters during the individual years of the study. In most of them, the variants without green pruning treatments (V_1) and those without manipulations on the green shoots – cluster thinning (V_3) and defoliation in the cluster zone (V_5) were characterized by significant higher absolute values, compared to the variants with shoot topping applied at different times – V_2 and V_4.

The degree of the shoots' maturation and their growth strength were directly correlated with the green pruning treatments and the weather, as the intensity of the impact of climatic conditions in the particular year was greater and stronger.

The growth strength of the vines in all variants was significantly greater in years with a wetter and cooler weather (2017 and 2020) and significantly less in medium-dry and very hot growing season (2019).

Shoot topping, as green pruning treatment, contributed to better maturation of the shoots, with the highest values, according to all reported indicators, being measured in the drier and hotter climatic conditions of 2019.

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AROMATIC AND ORGANOLEPTIC PROFILE OF WHITE WINES AGED IN OAK BARRELS

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ABSTRACT

A study was carried out on the influence of oak wood on the volatile aromatic and organoleptic profile of white wines. The study was focused on three white dry wines, purchased from the commercial network, from different producers and regions of Bulgaria. All samples were aged in barrels of various wood and different duration of contact. The studied wines showed a complex and diverse volatile composition and tasting properties due to the varietal features of the grapes, the soil and climatic conditions of the vintage and the region, the specific influence of the used method of contact with the wood. Riesling, 2021 vintage was found to have the highest ratio of total volatile compounds, total higher alcohols and terpenes. High levels of total esters were identified in Chardonnay, 2020 vintage. Main quantitatively dominant higher alcohols were 2-methyl-1-butanol, 3-methyl-1-butanol and 1-propanol, while esters were represented by ethyl acetate and isopropyl acetate. The aldehyde fraction was dominated by acetaldehyde in concentrations that positively affected fruit sensory. The terpenes linalool and geraniol were found in two of the studied wines – Riesling (2021) and Chardonnay (2020). Methanol was identified in all samples, with levels well below the acceptable threshold for white wines. The tested samples had different organoleptic characteristics in terms of color, aroma and taste, due to the diverse type and quantity of oak wood used, as well as the duration of the contact.

***Key words**: White wine, oak wood, volatile composition, organoleptic characteristics.*

INTRODUCTION

The use of oak wood in various forms is traditional in winemaking, because of the positive effect on the chemical composition and organoleptic qualities of the wine. This practice is increasingly being applied by producers in technological schemes for producing white wines, as a result of which they acquire a specific color, aroma and taste (Kyraleou et al., 2016; Botha et al., 2020; Zamora, 2022).

The oak wood species has a significant impact on the quality properties of the aging drinks. The differences in its structure and parameters are determined by the region of growing, the method of processing (drying and toasting), as well as the form in which it is used in production (Comfort, 2009; Cabrita et al., 2012; Herrero et al., 2016).

As a result of ongoing intensive extraction, oxidation-reduction, condensation and other processes, a number of compounds (mainly phenolic and volatile substances) pass from the wood to the wine, changing its chemical composition and sensory profile (Navojska et al., 2012; Nunes et al., 2017; Dumitriu et al., 2019). The concentration of volatile components (guaiacol, eugenol, syringic and vanillic aldehydes, methyloctalactone, furfural, methylfurfural ect.), which are released from the wood to the liquid depends on the oak botanical species and increases with the duration of contact (Baron et al., 2018; Loupassaki et al., 2018; Zamora, 2022).

Oak wood affects the wine taste in two ways. It might both improve its flavour length and make it softer through heterogeneous polymerization of tannins and contribute to wine astringency and bitterness. That depends on the manner of the wood treatment and the wine composition (Abrasheva et al., 2008).

Oak volatile components that have an impact on the wine aroma are mainly furfurals (dry fruit aroma), whiskey lactones (woody and coconut notes), eugenol (spices, cloves, smoke), 4-ethylphenol, 4-ethylguaiacol, vanillin and syringaldehyde, which have a low threshold of aroma perception (Navojska et al., 2012).

From an organoleptic point of view, the most important components passing from the wood are lactones (*cis*- and *trans*-β-methyl–γ-octolactone), associated with coconut aroma. Guaiacol and 4-methylguaiacol, formed during the heat treatment of wood and the breakdown of lignin, impart a smoky flavor. Furfural and 5-methylfurfural are formed as a result of the thermal degradation of cellulose and hemicellulose during toasting and impart an almond aroma. Eugenol, which increases during toasting, is associated with an aroma of spices and cloves. The concentration of the components extracted from the wood increases proportionally to the aging time (Dumitriu et al., 2019; Zamora, 2022).

Wines in contact with French oak with medium degree of toasting contain more volatile aromatic components and have a richer and more developed aroma compare to the American oak. French wood contributes to raising the levels of furan aldehydes. Furfural and guaiacol concentrations increase. The amount of *cis*- and *trans*- whiskey lactones is higher in American oak, but dropps down with increasing the wood toasting (Navojska et al., 2012). Depending on this, in sensory aspect, the American oak gives the wine pronounced aromas of vanilla, coconut, chocolate, while the French oak adds hazelnut, smokiness, spicy nuances of clove and cedar. The higher the toast level, the more coffee notes of mocha and espresso are perceived (Moore, 2021).

Herrero et al. (2016) studied the effect of toasting level and aging time on the volatile composition and sensory quality of Chardonnay and Sauvignon Blanc wines, aged in French oak barrels. The volatile compounds released by the oak wood into wines increased with the aging time except methylvanillate and vinylphenols. The concentration of vanillins, furfurals, guaiacols, eugenols and lactones increased over time. Their maximum quantities were found in medium toasting level barrels. Loupassaki et al. (2016) studied the evolution pattern of some selected wood-related volatiles and their impact on the aromatic profile of the wines produced by different aging techniques – barrel aging and use of wooden chips. Furfural was predominant substance and its concentration peaked after six months of aging, irrespective of the technique used. *Cis*-β-methyloctalactone tended to accumulate towards the end of the aging period. Its concentration was higher in the samples aged in the barrel made of American oak. Guaiacol and vanillin concentrations were low. The samples that received aging in barrels made of American oak had more intense aromatic profile.

The objective of this study was to determine the influence of oak wood on the volatile and organoleptic profile of Chardonnay and Riesling white wines.

MATERIAL AND METHODS

The study was carried out in 2023 at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria. Three white dry wines were the object of the test, purchased from the shops, from different producers and regions of Bulgaria. All wines were produced with oak wood, aged in a barrel with different duration of contact (Table 1).

The selected samples were subjected to a gas chromatographic analysis to identify the volatile components of the wine composition with and without aromatic influence and an organoleptic analysis to determine the sensory characteristics of the wines.

Aromatic profile

The main representatives from the group of esters, aldehydes, higher alcohols and terpenes were determined by GC-FID analysis in the studied wines.

The content of the main volatile aromatic compounds was identified on the basis of a stock standard solution prepared in accordance with the IS 3752:2005 method, comprising the following compounds (purity>99.0%): acetaldehyde, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol. 1-octanol was used as an internal standard.

The resulting standard solution was injected in an amount of 2 µL into a gas chromatograph Varian 3900 with a capillary column VF max MS (30m, 0.25mm ID, DF= 0.25µm), equipped with a flame ionization detector (FID) and carrier gas helium (He). Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manual, using a microsyringe.

The gas chromatographic determination parameters were: injector temperature – 220°C, detector temperature – 250°C, initial furnace temperature – 35°C/ retention for 1 min, rise to 55°C with a step of 2°C/min for 11min, rise to 230°C with a step of 15°C /min for 3 min. Total time of chromatography – 25.67 min.

After establishing the retention times of the compounds in the standard solution, the identification and quantification of the volatile compounds in the wine followed. The samples were previously distilled and the volatile composition was determined by injecting the distillate, in an amount of 2 µL, into the gas chromatograph.

Table 1. White wines produced in contact with oak wood, from different producers and regions of Bulgaria (according to data provided by the producer).

Sample №	Variety	Vintage	Region of production	Oak wood contact conditions
1	Chardonnay	2020	Southern Bulgaria	the wine was aged in new Bulgarian oak barrels, with a medium degree of toasting; duration of contact 24 months
2	Chardonnay	2021	Northern Bulgaria	the batch was a combination of wine where 33.3% was aged in Bulgarian oak barrels and 66.7% was aged in French oak barrels; the barrels had a light and medium degree of toasting; duration of contact 6 months.
3	Riesling	2021	Northern Bulgaria	the batch was a combination of wine where 37.5% was aged in Bulgarian oak barrels and 62.5% was aged in French oak barrels; the barrels had a light and medium degree of toasting; duration of contact 6.5 months

Organoleptic characteristics and tasting evaluation of the wines

A tasting panel of nine members from the IVE – Pleven participated in the organoleptic analysis. The samples were evaluated on a 100-point scale, according to the indicators of color, aroma, taste and general impressions. When processing the results, the highest and lowest obtained scores were not taken into consideration. To define the aromatic and taste characteristics of the wines, as a result of their contact with oak wood, the method of the main characteristics was used, through descriptive analysis (Prodanova, 2007).

3. Statistical processing of the results.

Statistical processing was made on the data obtained from triplicate performance of the chemical analyses, represented by mean value and standard deviation (±SD). The program Excel 2007 (Microsoft Office) was used for the determination.

RESULTS AND DISCUSSION

1. Determination of the volatile and aromatic composition of white wines from different producers and regions aged in oak barrels.

The data from the gas chromatographic analysis to identify the volatile compounds in the studied samples were presented in Table 2.

According to the total concentration of volatile compounds in white wines, it was the highest in Riesling (317.38±53.32 mg/dm^3). The content of total volatile compounds in Chardonnay, 2020 vintage was close to it (255.24±26.74 mg/dm^3), and the least amount of volatile components was found in Chardonnay, 2021 vintage (164.82±19.12 mg/dm^3).

The total concentration of higher alcohols followed the same order, despite the fact that the differences between the three wines were smaller. The highest content of higher alcohols was identified in Riesling (77.29±25.31 mg/dm^3), and the lowest in Chardonnay, 2021 vintage (53.78±5.09 mg/dm^3). Higher alcohols are products of yeast metabolism. Their quantitative accumulation in young wines is due precisely to this biological synthesis. Yeasts belonging to *Saccharomyces* metabolize grape sugars and amino acids to a wide range of varietal and quantitative range of higher alcohols (Bell and Henschke, 2005). In white wines, they present in a concentration of up to 150.00 – 400.00 mg/dm^3 (Gómez-Miguez et al., 2007; Chobanova, 2012). Depending on their ratio availability, they might have a positive effect on wine aroma by enhancing fruity and floral nuances, but in higher levels they imparte a negative aromatic effect related to notes of fusel oils and solvent (González-Alvarez et al., 2011; Cameleyre et al., 2015).

It is claimed that a higher alcohol content below 300.00 mg/dm^3 has a positive effect on the wine aroma (Gómez-Miguez et al., 2007), while amount above 400.00 mg/dm^3 has a strong negative influence (Rapp and Vesrini, 1991). The obtained concentrations of higher alcohols in the studied white wines were within the threshold concentration reflecting their positive impact on the aromatic quality of the wines.

Main representatives of higher alcohols identified in all three white wines were 2-methyl-1-butanol, 3-methyl-1-butanol and 1-propanol.

The highest content from the higher alcohol fraction was shown by 3-methyl-1-butanol. The highest quantity was found in Chardonnay, 2020 vintage (50.46±3.24 mg/dm^3). In the other two samples, its concentration did not differ significantly. Its amount could reach 200.00 – 500.00 mg/dm^3 (Chobanova, 2012). 3-methyl-1-butanol is a higher alcohol that has a strong influence on the formation of fruity aromatic nuances and shows high response to the acids in wine. That causes esterification to occur during the storage and aging process of the wines, complicating their aroma by this mechanism (Cometto-Muñiz and Abraham, 2008; Cameleyre et al., 2015).

2-methyl-1-butanol was found in lower concentrations, but the trend among the three studied white wines was similar to that for 3-methyl-1-butanol. Thus, 2-methyl-1-butanol was determined in the highest amount in Chardonnay, 2020 vintage (7.25±1.10 mg/dm^3). In the rest of the samples, its content was close. Usually that compound is found in wines in an average rate of about 36.00 mg/dm^3 (Chobanova, 2012). Its aromatic influence is associated with fruity nuances and a smoky aroma (Carpena et al., 2021; Karabagias et al., 2021).

The third major component identified was 1-propanol. Its concentrations differed between the three wines. Its presence (10.65±2.03 mg/dm^3) in the Riesling sample (2021) was the most significant. Chardonnay (2021) was the second one (4.23±0.94 mg/dm^3), and very low levels of this alcohol (0.98±0.02 mg/dm^3) were found in Chardonnay (2020). Its specific aromatic expression includes bitter notes (Zhang et al., 2015). It is also very reactive and forms esters with acetic, propionic and caprylic acids (Chobanova, 2012). 1-propanol was found as the main higher alcohol in 25 single-varietal white wines (Xarel.Lo variety) from different vintages of the Catalonia region, Spain (Mũnoz-Gonzalez et al., 2011), with its concentration ranging from 22.78 to 57.93 mg/dm^3. 1-propanol is reported to be present in amounts from 11.00 to 125.00 mg/dm^3 in wines from various countries (IARC, 1987).

In the samples 1-butanol and 2-butanol were also identified. 1-butanol was determined in two of the investigated wines – Riesling (2021) and Chardonnay (2021). Its amount in the first one (36.51±8.43 mg/dm^3) exceeded over twice that found in the second one (14.35±1.30 mg/dm^3). That compound is available in wines in a wide concentration range from 1.00 to 64.00 mg/dm^3 (Chobanova, 2012). 2-butanol was found only in the Chardonnay sample (2020) at concentration of 8.40±0.00 mg/dm^3.

The highest amount of total esters in white wines was identified in Chardonnay, 2020 vintage – 110.27±20.45 mg/dm^3. It was significantly higher than that analyzed in the other two samples, respectively three times that of Riesling (2021) and six times, comparing it to Chardonnay (2021).

Esters are a component of the volatile composition with a high aromatic influence, which is due to their low perception thresholds. They accumulate in the wine by several mechanisms: direct passage from the grapes (their amounts are very low – 10.00 – 30.00 mg/dm^3), accumulation during alcoholic fermentation (biological metabolic synthesis by yeast) and during long-term aging of the wines (through the process of esterification – a chemical reaction between higher alcohols and the acids in the wine) (Chobanova, 2012). In young wines, the total ester concentration ranges from 200.00 to 500.00 mg/dm^3 (Chobanova, 2012). The amount of total esters found in the studied white wines correlated with that range.

Table 2. Volatile and aromatic compounds in white wines made in contact with oak wood, from different producers and regions in Bulgaria.

Wine / Compounds / mg/dm^3	Chardonnay 2020 /Southern Bulgaria/	Chardonnay 2021 /Northern Bulgaria/	Riesling 2021 /Northern Bulgaria/
Acetaldehyde	70.00±0.00	92.59±12.24	197.50±8.25
Methanol	7.59±1.92	1.17±0.39	5.26±1.06
2-methyl-1-butanol	7.25±1.10	3.94±0.62	3.29±0.66
3- methyl-1-butanol	50.46±3.24	30.67±2.03	26.84±4.88
4- methyl-1-pentanol	-	0.59±0.20	-
1-propanol	0.98±0.02	4.23±0.94	10.65±2.03
1-pentanol	-	-	-
1-butanol	-	14.35±1.30	36.51±8.43
2-butanol	8.40±0.00	-	-
1-hexanol	-	-	-
2-phenylethanol	-	-	-
Total higher and aromatic alcohols	67.09±4.36	53.78±5.09	77.29±25.31
Ethyl acetate	92.57±20.41	15.22±1.17	21.88±12.70
Pentyl acetate	7.19±0.00	-	-
Propyl acetate	-	-	-
Isopropyl acetate	1.92±0.04	2.06±0.23	5.19±1.73
Isobutyl acetate	-	-	8.54±4.27
Phenyl acetate	-	-	-
Ethyl hexanoate	-	-	-
Ethyl butyrate	8.59±0.00	-	1.14±0.00
Ethyl decanoate	-	-	-
Total esters	110.27±20.45	17.28±1.40	36.75±18.70
Linalool oxide	-	-	-
Linalool	0.29±0.01	-	-
Nerol	-	-	-
Geraniol	-	-	0.58±0.00
Total terpenes	0.29±0.01	-	0.58±0.00
Total quantity	255.24±26.74	164.82±19.12	317.38±53.32

Main identified representatives of esters in the samples analyzed in this study were ethyl acetate and isopropyl acetate. Ethyl acetate was quantitatively dominant. It was present in the highest concentration (92.57±20.41mg/dm^3) in Chardonnay, 2020 vintage. In the other two wines, it was found in lower amounts, respectively 21.88±12.70 mg/dm^3 (Riesling, 2021 vintage) and 15.22±1.17 mg/dm^3 (Chardonnay, 2021 vintage). Ethyl acetate is a dominant ester characterized by a dual effect on wine aroma. It is synthesized in young wines mainly biologically (by yeast). In concentrations of 80.00 to 100.00 mg/dm^3 it positively affectes the aroma. Above these rates, the wines acquire a characteristic unpleasant acetic acid and chemical scent (Plata et al., 2003). Its concentrations in our studied white wines were within the range that characterized its positive influence on the aromatic quality.

The second ester found in all white wines studied was isopropyl acetate. It was in the highest concentration (5.19±1.73 mg/dm^3) in Riesling (2021). Its concentration in the other two samples was very close, respectively 2.06±0.23 mg/dm^3 (Chardonnay, 2021) and 1.92±0.04 mg/dm^3 (Chardonnay, 2020). Usually that ester imparts a banana aroma to the wines (Carpena et al., 2020).

Isobutyl acetate was identified only in Riesling (2021) at a concentration of 8.54±4.27 mg/dm^3. This ester is normally available in wines in an amount of 1.00 to 10.00 mg/dm^3 (Chobanova, 2012). The content found in this study was in correlation with this range. Isobutyl acetate imparts a fruity aroma to the wines (Carpena et al., 2021).

Ethyl butyrate was determined in two of the investigated white wines – Chardonnay (2020), where its concentration (8.59±0.00 mg/dm^3) was higher, and Riesling (2021) (1.14±0.00 mg/dm^3). This ester is a metabolite of the *Saccharomyces* yeast. Selected strains might produce it up to 20.06±2.23 mg/dm^3 (Ma et al., 2020). It was found in concentrations up to 0.75 mg/dm^3 in French wines by Antalick et al. (2014). The ester was also identified as the main one in Marquette and Frontenac variety wines from North Dakota, USA (Rice et al., 2018).

Acetaldehyde was the only aldehyde identified. It was found in the highest amount (197.50±8.25 mg/dm^3) in Riesling (2021), followed by Chardonnay (2021) (92.59±12.24 mg/dm^3), and the lowest in Chardonnay (2020) (70.00±0.00 mg/dm^3). It represents 90% of the total aldehydes in wines, and its concentration varies widely from 10.00 to 200.00 mg/dm^3. The higher content in dry wines could serve as evidence of over sulphitation. It is specific for this aldehyde that in samples with concentrations up to 130.00 mg/dm^3 it gaves a fruity character to the wines, at higher levels, however, it causes the appearance of oxidized tones in the aroma (Noble et al., 1987; Ribéreau-Gayon et al., 2000).

Terpenes were identified in two of the white wines studied. The total terpenes content of Riesling, 2021 vintage (0.58±0.00 mg/dm^3) was higher than that found in Chardonnay, 2020 vintage (0.29±0.01 mg/dm^3). In Chardonnay, 2021 vintage, no terpenes were identified. Among the representatives of the terpene fraction, one terpene was identified in the other wines. Geraniol and linalool in Riesling (2021) and Chardonnay (2020), respectively.

The content of methyl alcohol was analyzed in all three studied wines. It is a normal component of wine volatile composition resulting from the hydrolysis of pectin in the fruit by the pectolytic enzyme complex. Its detected levels in the three white wines were very low. Comparing them, its concentration was the highest in Chardonnay, 2020 vintage (7.59±1.92 mg/dm^3), and the lowest in Chardonnay, 2021 vintage (1.17±0.39 mg/dm^3).

Stegarus et al. (2021) investigated the effect of barrel aging and the addition of oak chips on the volatile composition of Chardonnay wines. They found that in the initial stages of contact with wood, the extraction of carboxylic, phenolic and volatile acids was accelerated. The extraction of furan aldehydes, oak lactones, phenol aldehydes and alcohols depended mainly on the alcohol content of the wine and less on its pH. The higher alcohols as isobutanol, isopentanol and 2-phenylethanol were found in the highest ratio. After three months of aging, only the amount of isobutanol decreased, while that of the other two higher alcohols went up. A drop down was also observed in the other higher alcohols present in minimal concentrations – 4-methyl-1-pentanol, 2-nonalol, 1-heptanol, 3-methylthio-1-propanol. The main determined esters were the ethyl esters, with a predominance of ethyl lactate, ethyl caproate and ethyl octanoate. From the group of acetate esters, isoamyl acetate was predominant. During aging, most of the esters increased in concentration. Butyrolactone showed the highest concentration from the lactones. When aging with wood, the amount of volatile phenols in the wine went up (p-vinylguaiacol, acetovanillone).

2. Determination of the sensory characteristics of white wines from different producers and regions aged in oak barrels.

The contact of wine with wood has a beneficial effect on its sensory features. As a result of the ongoing processes and transformations, the clarity improves, the aroma and taste become more developed and complex. That depends to a significant extent on the type of wood and the way it is processed (Vilela et al., 2014; Nunes et al., 2016; Naranjo et al., 2021; Zamora, 2022).

The results from the sensory analysis of the studied samples were presented on Figure 1. The studied white wines had similar color characteristics. All wines were of good clarity and color tint corresponding to the vintage. The samples Chardonnay and Riesling, 2021 vintage, showed better shine and the color intensity was the most pronounced in the Chardonnay, 2020 vintage and Riesling, 2021 vintage.

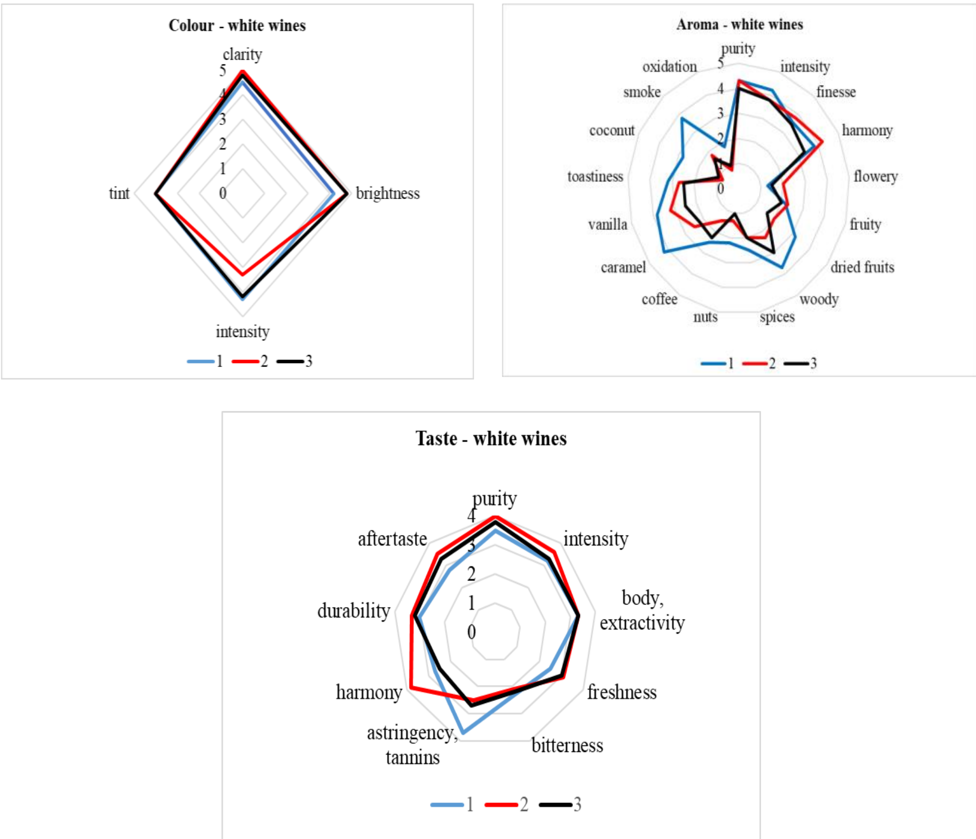

Figure 1. Organoleptic profile of oak barrel-aged white wines from different producers and regions

(Legend: 1 – Chardonnay, 2020 vintage, 2 – Chardonnay, 2021 vintage, 3 – Riesling, 2021 vintage)

More significant differences between the samples stood out in their aroma. Chardonnay, vintage 2020, had the best aromatic qualities. The aroma of this wine had the best harmony, with enhanced woody and smoky notes, as well as dried fruit, caramel, vanilla. That was due to the contact of the wine with the wood – aging in a new Bulgarian oak barrel for 24 months. In the samples from Danube Plain region, Chardonnay showed a more harmonious aroma with more pronounced fruit and vanilla nuances, as well as toastiness and smoke. The Riesling sample had stronger woody aromas and nuts, resulting from the slightly longer contact with the wood.

The three white wines had similar taste characteristics in terms of purity, intensity, density and persistence. Chardonnay, 2020 vintage, had a less pronounced freshness and aftertaste, but a more pronounced tartness and tannin compared to the other two samples. Chardonnay, 2021 vintage was characterized by the best balance in taste. In Riesling, 2021 vintage, none of the descriptor indicators stood out.

A number of authors point out that not only the type and the wood processing, but also the form under which it is used, the dose, the time of application and contact, are important for the sensory profile of the wines.

In terms of sensory results, contact with oak enhances the “woody aroma” of the wines, the descriptors for “body” and “astringency” are more intrusive and intense (Galdo et al., 2019).

Botha et al. (2020) investigated the influence of medium-toasted French oak barrels on the sensory profile of South African white Chenin Blanc wines fermented and aged in contact with wood for 4 and 9 months. The control had preserved the fruitiness in the aroma, while the samples in contact with the wood lost it and acquired an aroma associated with vanilla, oak, marmalade, dried apple and quince, coffee, caramel, honey, tobacco, smoke. The roasted coffee and tobacco notes were due to furfurylthiol and the board aroma came from the concentration of trans-2-nonenal, trans-2-octanal and 1-decanal.

In white Verdejo wines, Petrozziello et al. (2020) investigated the effect of using medium-toasted oak chips during the alcoholic fermentation and aging. Compared to the control, in the samples that were in contact with wood, descriptors such as freshness, green apple, fruitiness were reduced, and the intensity of notes such as dried fruit, coconut, sweet spices, toastiness was increased.

CONCLUSION

From the study carried out, it could be summarized that the investigated wines showed a complex and diverse volatile composition and organoleptic profile due to the varietal specifics of the grapes, the soil and the weather conditions of the specific harvest and region, the specific influence of the used method of contact with oak wood. Based on this, the following conclusions could be drawn:

Riesling wine, 2021 vintage, was characterized by the highest content of total volatile compounds, as well as total higher alcohols and total terpenes. High rates of total esters were identified in the 2020 vintage Chardonnay.

Main quantitatively dominant higher alcohols were 2-methyl-1-butanol, 3-methyl-1-butanol and 1-propanol, while esters were represented by ethyl acetate and isopropyl acetate.

Acetaldehyde in concentrations that positively affected the fruit sensory dominated in the aldehyde fraction.

Terpenes were identified in two of the investigated white wines – Riesling (2021) and Chardonnay (2020). They were typically represented by linalool and geraniol.

Methanol was found in all wines studied, and its levels were low, well below the threshold of its permissible presence in white wines.

The investigated white wines had different sensory characteristics in terms of color, aroma and taste, due to the different species and amount of oak wood used, as well as the duration of the contact.

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SECTION 2

SUSTAINABLE AND PRECISION AGRICULTURE

UDK: 631.147(497.7)

STATUS AND VISIONS FOR THE DEVELOPMENT OF ORGANIC PRODUCTION IN RN MACEDONIA

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ABSTRACT

In 2020, the European Commission published its 'Farm to Fork' strategy – "for a fair, healthy and environmentally friendly food system" – along with the EU biodiversity strategy, where 25 % of the EU's agricultural land should be under organic farming by 2030. Although some of the EU countries even now have exceeded or are quite close to the specified percentage (Liechtenstein, Austria and Estonia), the plan is quite ambitions and means that by 2030 the EU should triple its organic land area (compare with 2019), increase its overall CAP expenditure 3-5-fold by 2030, and dedicate 9-15% of the CAP budget to organic (instead of 1.5% as it now and the 5% forecasted for the 2023-2027 period). In RN Macedonia, organic farming represents 0.83% of total arable land, well below the 2% target set by the National Strategy for Agriculture and Rural Development (2021 - 2027), and the 4% envisioned in the National Plan for Organic Production (2013 - 2020). Therefore, significant reforms are necessary. These include: increasing farm productivity, raising education and awareness, strengthening political support and subsidies, expanding market development, promoting technical support and innovation, protecting biodiversity, adapting to climate challenges, and fostering international cooperation and partnerships. If these measures are properly coordinated and implemented, the country can achieve its organic farming objectives to meet at least 2% organic arable land goal by 2027.

Key words: *Organic, productivity, education, cooperation, market.*

INTRODUCTION

According to the guidelines for organic food in the *Codex Alimentarius* by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), organic agriculture is defined as "a holistic production management system that promotes and enhances the health of agro-ecosystems, including biodiversity, biological cycles, and soil biological activity. It prioritizes management practices over the use of off-farm inputs, recognizing that regional conditions require locally adapted systems. This is achieved, where possible, through agronomic, biological, and mechanical methods rather than synthetic materials to fulfil any specific function within the system." In addition, organic agriculture should be based on the following four fundamental principles: appropriate design and management of biological processes based on ecological systems that utilize natural resources, limited use of external inputs, strict restriction of the use of chemically synthesized substances, and adaptation, where necessary, of organic production rules, taking into account sanitary conditions, regional climate differences, local conditions, development stages, and specific agricultural practices.

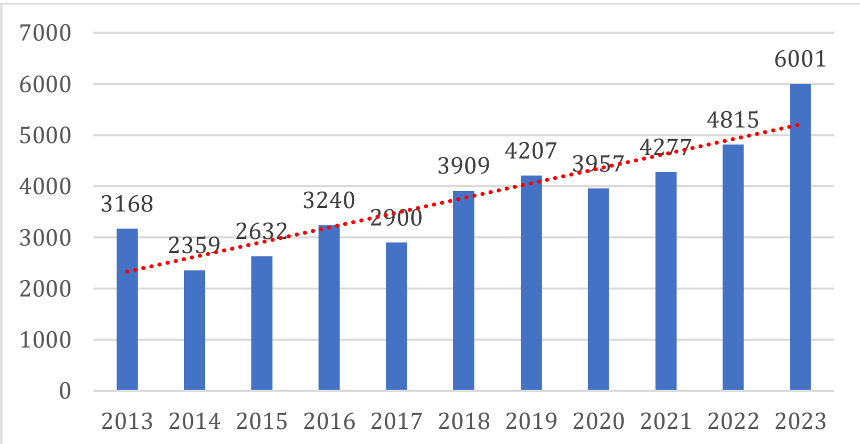
Organic agriculture production worldwide and in EU

In 2022, globally, 96.4 million hectares were under organic agricultural production, representing 2.0 percent of the total agricultural land. Compared to 2021, the area under organic agriculture increased by 26.6 percent, or by 20.3 million hectares. The organic food and beverage market reached nearly €135 billion in 2022, with leading countries being the United States (€58.6 billion), Germany (€15.3 billion), and China (€12.4 billion) (FiBL & IFOAM, 2024). The demand for organic agricultural products in Europe, particularly within the EU, has been steadily increasing in recent years, with the organic food market almost doubling between 2015 and 2020. According the report by the Directorate-General for Agriculture and Rural Development (2021), 9.9% of the total arable land in the EU was under certified organic production (Organic Action Plan: Action Plan for the Development of Organic Production, 2023). Four countries: France, Spain, Italy, and Germany, account for 59% of the total organic areas. However, in relative terms at the national level, member states such as Liechtenstein lead with organic agriculture covering 43% of the country's total arable land. Austria, Estonia, and Sweden follow with more than 20%. As part of the European Green Deal, the EU's "Farm to Fork" (F2F) food policy aims to have 25% of agricultural land under organic farming by 2030. The plan is quite ambitions and means that by 2030 the EU should triple its organic land area (compare with 2019), increase its overall CAP expenditure 3-5-fold by 2030, and dedicate 9-15% of the CAP budget to organic (instead of 1.5% as it now and the 5% forecasted for the 2023-2027 period (Common Agricultural Policy (CAP) - IFOAM Organics Europe). The EU's organic product market is valued at €44.9 billion annually, representing 37% of the global organic food market, placing the EU second after the US, which accounts for 41% (Bozic et al., 2024).

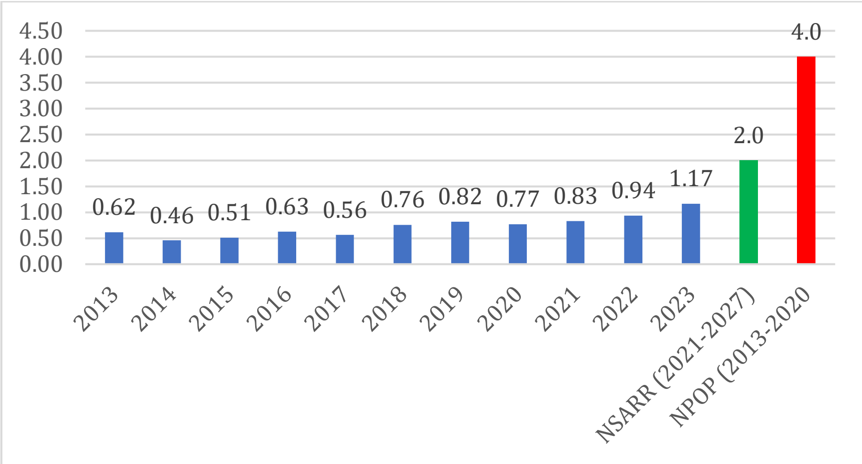
Organic agriculture production in the Republic of North Macedonia

According to the latest data, in 2023, there were 6,001 hectares of certified organic production (Graph 1), representing 1.17% of the total arable land in 2023 (514,375 hectares) (MAFWE, 2023). This figure still falls short of one of the specific goals outlined in the National Strategy of Agriculture and Rural Development (2021–2027), which aims for the share of total organic area in total arable land to reach 2% i.e. from the National plan for organic production where the share of organic areas was planned to amount 4% (Graph 2).

Organic production is supported in accordance with the Law on Agriculture and Rural Development under two pillars of measures: i) for agriculture and ii) for rural development. The total amount of funds paid to beneficiaries in the program period from 2015 to 2023 amounts to 12.3 million euros. From year to year, the amounts for support are increasing, which is expected to lead to an increase in both production and the number of producers who choose this type of production. The financial support for organic agricultural production for 2023 amounts to a total of 104,000,000.00 MKD (1.68 million euros or 1.6% of the total financial support for agriculture) (Official Gazette of the Republic of North Macedonia, No. 282/22).



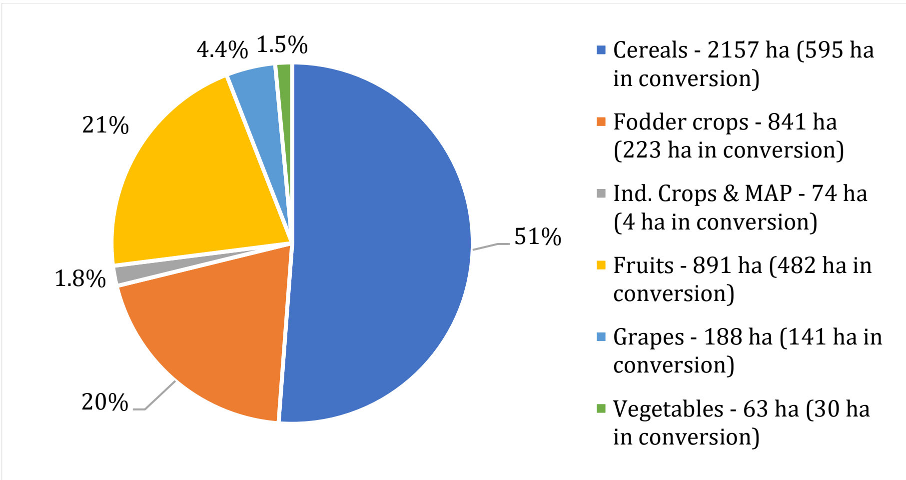
Graph 1. RN Macedonia: The area under organic production/ha, 2013 - 2023



Graph 2. The area under organic cultivation in total arable land in %, 2013 - 2023

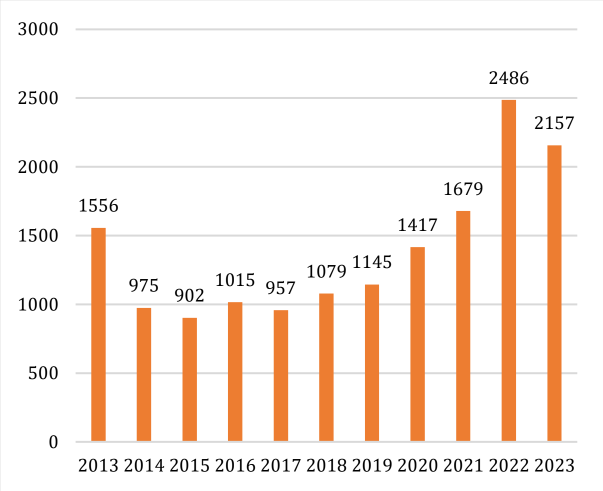
Organic crop production in RN Macedonia

Cereals occupy the largest share of the total certified organic area, followed by fruit and fodder crops (Graph 3) (**MAFWE, 2023; SSO, 2023).**

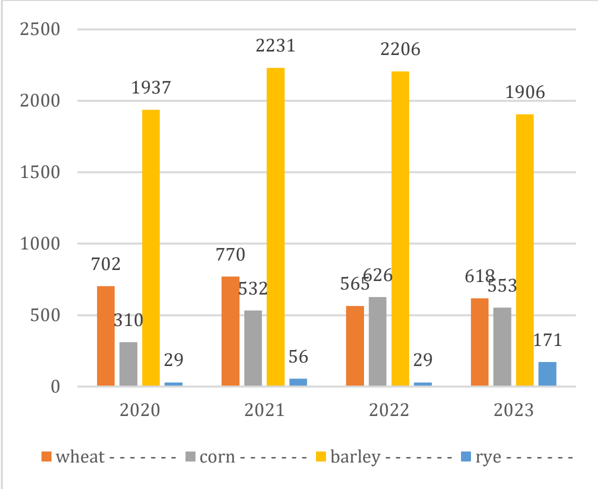


Graph 3. Area of organic crop production, 2023

Out of the total 2752 hectares, 2157 hectares are certified cereal crops, while 595 hectares are in the conversion period. This marks a decrease of 329 hectares compared to 2022 (Graph 4). The main organic cereal crop is barley, with an annual production of 1,906 tons, accounting for 52% of the total organic cereal production (3,653 tons) (Graph 5) (SSO, 2023).

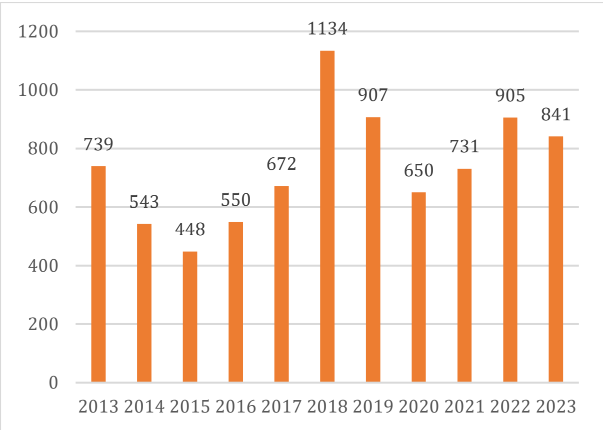


Graph 4. Cultivated area under organic cereals/ha, period 2013 - 20123

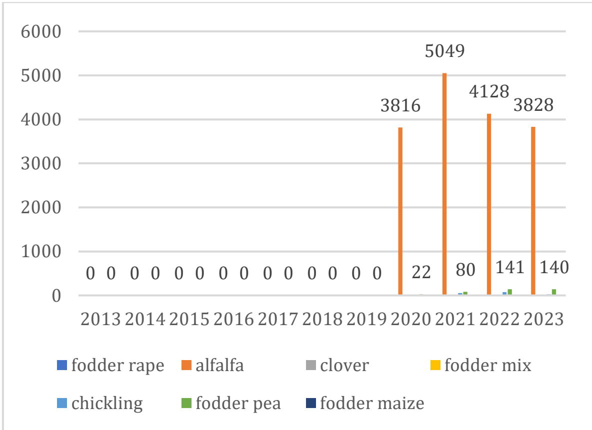


Graph 5. Organic production of cereals/t

The largest areas under organic forage crops were recorded in 2018 - 1,134 hectares. In 2023, this area decreased by 35% to 841 hectares (Graph 6), representing 2% of the total forage crop area (41,647 hectares). The main organic forage crop is alfalfa, with a production of 3,828 tons, which is an 8% decrease compared to 2022 and a 32% decrease compared to 2021 (Graph 7). Alfalfa accounts for more than 95% of the total organic forage crop production (4,021 tons) (SSO, 2023).



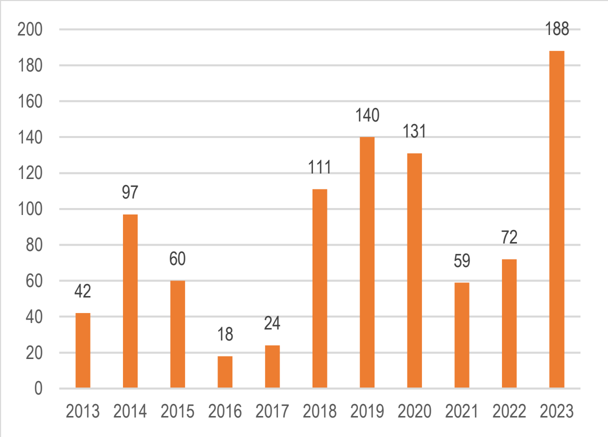
Graph 6. Cultivated area under organic forage crops/ha



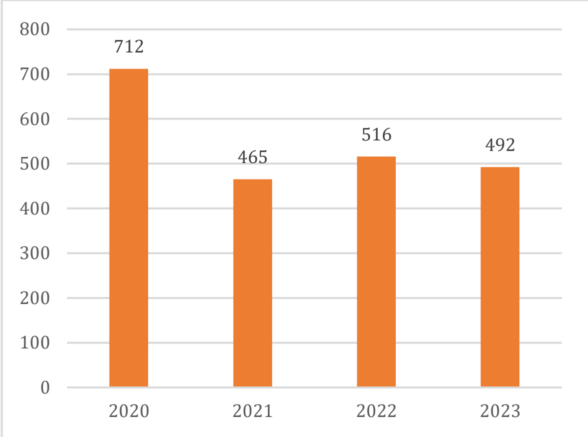
Graph 7. Organic production of forages/t

The areas under organic orchards have been continuously increasing, reaching their peak in 2023 at 891 hectares, or about 5.4% of the total orchard area in the country (16,423 hectares), with various organic fruit species represented. The largest areas are dedicated to almonds (201 hectares), followed by sour cherries (126 hectares), hazelnuts (111 hectares), and plums (95 hectares) (Graph 8). In terms of production, the highest yield was achieved with organic apples -- 422 tons, accounting for 26% of the total organic fruit production (1,602 tons), followed by plums with 252 tons (15.7%), sour cherries with 150 tons (9.4%), and peaches with 142 tons (8.8%) (Graph 9) (SSO, 2023).

The area under organic vineyards shows an increasing trend, reaching 188 hectares in 2023 (Graph 10), or 0.8% of the total vineyard area (22,353 hectares). The total annual production of organic grapes in 2023 was 492 tons, which is 45% less than the record 712 tons produced in 2020. Most of this production is used for making organic wine (Graph 11) (SSO, 2023).

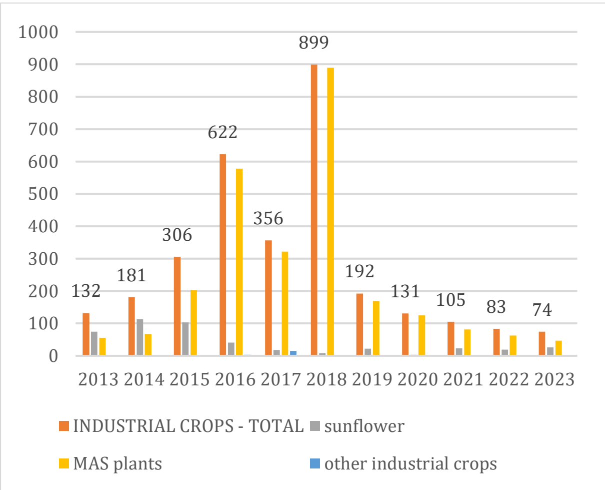


Graph 10. Cultivated area under organic vineyards/ha

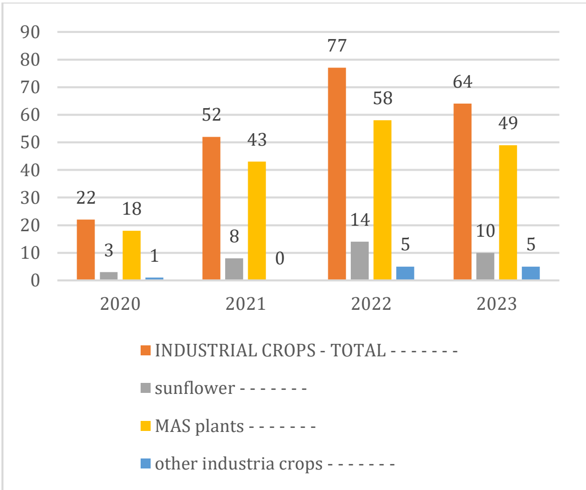


Graph 11. Organic grapes production/t

The certified area under organic industrial crops has experienced a significant decline, considering that in 2018 these crops were grown on 899 hectares. In 2023, they are cultivated on 74 hectares, of which 47 hectares, or nearly 64%, are dedicated to medicinal, aromatic, and spice plants; 26 hectares to organic sunflower; and 1 hectare to other industrial crops (Graph 12). Of the total production of 64 tons, 49 tons come from medicinal, aromatic, and spice plants, 10 tons from organic sunflower, and 5 tons from other industrial crops (Graph 13).

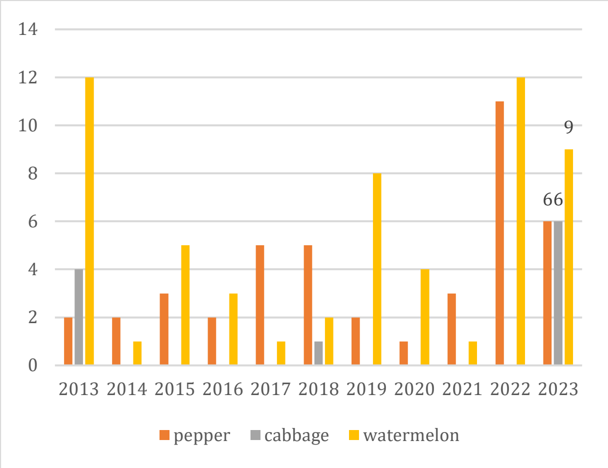


Graph 12. Cultivated area under organic industrial crops/ha

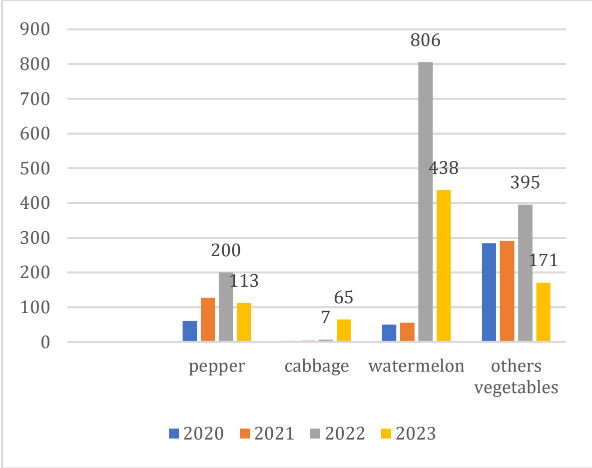


Graph 13. Organic production from industrial crops/t

From the total area of vegetable crops - 50,995 hectares, organic vegetable crops occupy only 62.9 hectares. The largest areas are dedicated to watermelon (9 hectares), cabbage (6 hectares), and peppers (6 hectares) (Graph 14). The total production amounts to 872 tons, distributed as follows: 438 tons of watermelon, 113 tons of peppers, 65 tons of cabbage, and 171 tons of other vegetable crops (Graph 15) (SSO, 2023).



Graph 14. Cultivated area under organic vegetable crops/ha



Graph 15. Production of organic vegetable crops/t

Opportunities which encourage the organic production in the Republic of North Macedonia

North Macedonia offers numerous opportunities to encourage organic production, driven by its untapped potential in both domestic and international markets. Some of them are following:

- export potential: organic products from North Macedonia can be marketed to the growing European organic product market.
- synergy with tourism: rural, eco-tourism, and commercial tourism in North Macedonia provide opportunities to promote and create markets for locally produced organic products.
- willingness of farmers to engage in organic farming: this is especially evident among young farmers who are eager for innovation and to adopt knowledge based on organic principles and practices.
- production of specific and high-value organic plants i.e. products: agricultural producers can specialize in the production of less commercial plants and products that have high value and are in demand both domestically and on foreign markets (e.g., organic production of artichokes, broccoli, and medicinal plants in Crnicani - Dojran).
- contract farming: possibility of contract farming between organic producers and buyers/processors of organic products, benefiting both parties: producers will have a guaranteed market for their products, and buyers will know that the purchased goods meet required ecological standards (e.g., organic pepper production in Bitola, Prilep, and Sveti Nikole).
- incorporation of fallow land into organic farming: according to the Ministry of Agriculture, Forestry and Water Economy (MAFWE), out of 6001 ha of organic production, 1536 ha are fallow. Utilizing these areas could significantly contribute to an increase in organic production and create new job opportunities.
- education and research: in the country, there are four higher educational and research institutions: the Faculty of Agricultural Sciences and Food in Skopje, the Agricultural Faculty in Štip, the Institute of Agriculture in Skopje, and the Institute of Animal Husbandry in Skopje, which include organic production in their curricula (first and second cycles of studies) and target specific issues related to organic farming in their research projects.

Vision for the development of organic production in the Republic of North Macedonia

The development of organic agriculture in North Macedonia should be envisioned as an opportunity and potential for contributing to sustainable economic growth, minimizing risks to human health, and serving as a fundamental factor in preserving and protecting the environment. To address challenges and capitalize on opportunities, the following components must be incorporated into the vision for the development of organic production and integrated into the strategy for developing organic agriculture:

Improvement of farm productivity through:

promotion of crop rotation, green fertilization, and other sustainable measures (to increase yields and soil fertility).

intensify cooperation between the scientific community and organic producers to adopt new technologies and skills.

Education and awareness raising:

farmer education: conduct trainings and workshops on modern organic farming techniques, effective resource management, and the application of biodiversity principles.

consumer awareness: promote the benefits of organic food through targeted campaigns, media outreach, and strategic marketing initiatives.

Improving political support and subsidies:

financial incentives: enhance state support by providing subsidies for organic producers to cover certification costs, adopt new technologies, and implement innovative practices.

regulatory framework: strengthen legislation related to organic agriculture, ensuring easier access to information and certification processes.

Improving market channels:

local markets and distribution: develop new market opportunities, such as local markets and direct farmer-to-consumer sales (e.g., farmer's markets), to provide organic producers with easier access to consumers.

export opportunities: promote the export of organic products by enabling trade with countries that have high demand for organic food.

Technical support and innovation:

investments in research and development: encourage projects aimed at developing new techniques for organic production and improving existing methods.

technology: promote the use of innovative technologies for efficient management of water resources, soil, and plant protection, eliminating the need for synthetic pesticides and chemical fertilizers.

Protection of biodiversity:

preservation of indigenous species: encourage the use of local varieties and indigenous species that are naturally adapted to the pedo-climatic conditions in the country.

sustainable soil management: implement agroecological practices to enhance and protect natural soil resources and promote biodiversity within the rhizosphere.

Climatic adaptations:

adapting to climate change: develop strategies to address climate change by utilizing organic production methods that conserve soil, reduce erosion, and mitigate land degradation.

International cooperation and partnerships:

programs for international cooperation: participate in international networks for organic agriculture and leverage international funding opportunities for development.

collaboration with NGOs and experts: foster stronger partnerships with non-governmental organizations, scientific institutions, and experts in the field of organic agriculture.

By implementing these recommendations, North Macedonia has the opportunity to overcome challenges and achieve sustainable growth in the organic agriculture sector and, by the end of 2027, the organic production to participate with 2% in structure of agricultural production in the country.

CONCLUSION

The Republic of North Macedonia has considerable potential for organic agriculture. Despite the all-optimistic indicators, organic farming and the production of organic food still face many challenges. The main barriers to the slow growth and better positioning of organic agriculture must be sought in improved cooperation between various national and international stakeholders and the promotion and exchange of knowledge about the advantages of organic farming, as well as more intensive marketing campaigns that would improve the positioning of organic agriculture and increase the consumption of organic products. Nevertheless, there are many opportunities to encourage this type of production in the country. Awareness of healthy nutrition, sustainable food production, and environmental protection is constantly growing worldwide. In light of the COVID-19 pandemic, consumers are increasingly concerned about their health, leading to rising demand for organic products. As a country where tourism is an important industry, the enormous potential for promoting organic agriculture must also be viewed through tourism and gastronomic offers, as well as through the development of new technologies that would further promote organic products not only within the country but also abroad.

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IMPLEMENTING GOOD AGRICULTURAL PRACTICES IN MUSHROOM CULTIVATION IN NORTH MACEDONIA

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ABSTRACT

Good Agricultural Practices (GAP) in mushroom production emphasize sustainable and efficient methods that promote healthy, high-quality yields. By properly implementing GAP, producers can reduce the risk of contamination, enhance food safety, and protect the environment through comprehensive soil and water management. To maintain the effectiveness of GAP, it is essential to review the results of these practices annually. This ongoing evaluation allows for continuous updating of plans and procedures, ensuring alignment with the latest agricultural standards. Key aspects of good farming practices in mushroom cultivation include the selection and reception of quality raw materials, proper pasteurization temperatures, optimal incubation and fruiting conditions, and rigorous hygiene protocols. It is crucial to prepare detailed procedures and record sheets for each critical control point, which should always be readily accessible. Additionally, the paper addresses the utilization of spent substrate after mushroom harvesting. This substrate not only serves as an excellent base for vermicomposting but can also enrich the soil, be used as firewood, and serve as a component in growing substrates in horticulture. Implementing Good Agricultural Practices facilitates traceability and control throughout all production stages, enhancing competitiveness and sustainability in the mushroom cultivation sector.

Key words: *good agricultural practice, circular economy, spent substrate, sustainable agriculture.*

INTRODUCTION

The desire for greater sustainability, improved food security through increased diversity, and the development of more reliable sources of income, especially for smallholder farmers, suggests that mushroom cultivation may be a viable option. Provides efficient and cost-effective biotechnology (Bradley, 2013), It can provide continuous growth with high biological efficiency (Jonathan et al., 2012).

In response to mounting threats to agricultural sustainability—ranging from environmental degradation and climate change to increasing concerns over hazardous chemical residues in food—Good Agricultural Practices (GAP) have emerged as a comprehensive global framework for ensuring food safety and system resilience, exemplified by Korea's adoption of GAP in 2006 as a leading certification standard that emphasizes traceability and rigorous monitoring of pesticide residues, heavy metals, and microbial contaminants to address escalating societal demands for health-conscious and transparent food production (Vijayakumar et al., 2021; Lee et al., 2017). The Code of Good Agricultural and Hygiene Practices outline sustainable agricultural methods tailored to regional climates and existing systems, ensuring optimal land use, crop rotation, food safety, environmental protection, and animal welfare through the integration of best practices and commonly applied agricultural technologies in the Republic of Macedonia (Code of Good Agricultural and Hygiene Practice, 2010). The purpose of the Code of Good Agricultural Practice is to establish the exact procedures in the agricultural production process that would minimize the threats of degradation and loss of this limited natural resource, and which relate to measures and procedures for preserving and improving soil fertility. (Guide to Good Agricultural Practices, 2010).

In 1997, the retailers who dominated the marketing of fresh fruit and vegetables in Europe came together to promote a program that minimizes threats to human health from fresh fruit and vegetables and products derived from them. They established the standards for Good Agricultural Practices (GAP). These standards, which were revised in 2007 and named GLOBALGAP, have been accepted by all countries in the world. According to these standards, retailers receive guarantees from producers and suppliers that the products they put on their supermarket shelves will not harm their customers (Kilic et al., 2020).

Good agricultural practices are usually regulated by individual regulations in each country and usually address the use of protection agents and aspects related to food safety. Considering the international dimension of trade in agricultural products, Good Agricultural Practices are also prescribed by international institutions such as the Food and Agriculture Organization of the United Nations – FAO (Angelovski, 2006). In the Republic of Macedonia, good agricultural practices are not set aside as a separate document, but are woven into the laws and regulations that regulate the various parts of agriculture (Angelovski, 2006). The first EUREPGAP (today GLOBALGAP) certificate for primary agricultural production was implemented from Sashko Todorov in the Republic of Macedonia and the Balkans with the certificate number ECAS 2005-3141-1, in the mushroom LABEKO (Filcak & Atkinson, 2006).

In the modern world, the cultivation of wild mushrooms and mushrooms is the only way to ensure food safety and fight malnutrition without affecting the environment, it is a step towards a non-green revolution. Relying on the green part of nature for plant fiber and protein can be reduced by practicing mushroom cultivation on a large scale. It is the best source for achieving zero hunger and no poverty for sustainable development goals (Niazi & Ghafoor, 2021). Mushroom cultivation, integrated into agricultural systems, offers environmental benefits such as improved resource efficiency, reduced waste, and enhanced soil fertility (Behera et al., 2004; Gangwar et al., 2013). By recycling agricultural waste into substrates, mushrooms reduce waste and enrich soil nutrients. Spent mushroom substrate (SMS), rich in nutrients, can serve as organic fertilizer or soil supplement, promoting sustainable agriculture and reducing reliance on synthetic fertilizers (Kushwaha, 2023).

This study explores mushroom cultivation technology, focusing on Good Agricultural Practices (GAP) to optimize processes and mitigate risks. It emphasizes managing critical points like raw material selection, temperature control during pasteurization, incubation, fruiting, strict hygiene, and sustainable use of spent substrate.

MATERIALS AND METHODS

This paper will cover the cultivation of the following mushrooms: Oyster Mushroom (*Pleurotus ostreatus*), Reishi (*Ganoderma lucidum*), Shiitake (*Lentinula edodes*), Lion's Mane (*Hericium erinaceus*), Maitake (*Grifola frondosa*) using Good Agricultural Practices. For the production of mushrooms, a pure culture is isolated. The mycelium is grown on Malt agar.

The implementation of GAP in mushroom cultivation not only improves the safety and quality of products, but also creates a management system that facilitates the monitoring, adaptation and improvement of agricultural practices. This contributes to the sustainability and competitiveness of the industry (UK Cooperative Extension Service, 2012).

In this study, these several mushrooms will be included:

Oyster mushroom (*Pleurotus ostreatus*)

Pleurotus ostreatus, commonly known as the oyster mushroom, belongs to the genus Pleurotus (family Pleurotaceae, order Agaricales, class Agaricomycetes, phylum Basidiomycota)

(Jacq. ex Fr.) (Kummer, 1871). They are valued for their rich mineral content, medicinal properties, short life cycle, and ability to recycle agricultural and industrial waste (Sibel Yildiz et al., 2002). Their substrate serves as a fertilizer, soil regenerator, and cattle feed post-harvest, offering solutions for waste disposal, economic benefits, and environmental protection (Brenneman et al., 1994; Soto-Cruz, 1999; Deepalakshmi & Sankaran, 2014).

Dabova Sjajnica (Ganoderma lucidum)

Ganoderma lucidum (W. Curt.: Fr.) P. Karsten belongs to the genus Ganoderma, family Ganodermataceae, order Polyporales, class Agaricomycetes, and phylum Basidiomycota (Miles & Chang, 2004). In Macedonia, six Ganoderma species are identified, including the widespread *G. applanatum* (found on beech stumps) and *G. lucidum* (on oak trunks) (Karadelev et al., 2008; Stojanović, 2016).

Shiitake (*Letinus edodes*)

Lentinus edodes (Berk.) Pegler, commonly known as shiitake, belongs to the genus Lentinula, family Omphalotaceae, order Agaricales, class Agaricomycetes, and phylum Basidiomycota. The sawdust block method, using a mix of oak sawdust, straw, corn, and additives, has become popular for cultivating high-quality shiitake (Miles & Chang, 2004). These mushrooms typically grow on deciduous trees like chestnut, oak, and beech (Wasser, 2004).

Lion's Mane (*Hericium erinaceus*)

Hericium erinaceus, commonly known as Lion's Mane, belongs to the genus Hericium, family Hericiaceae, order Russulales, class Agaricomycetes, and phylum Basidiomycota (Bull.) (Persoon, 1797). The fruit body is irregularly bulbous with spiny hymenophores and grows on dead or dying deciduous trees. It can be cultivated on sterilized beech sawdust supplemented with wheat bran or corn flour, yielding high production (Kirchhoff, 1996; Siwulski & Sobieralski, 2007).

Maitake (*Grifola frondosa*)

Maitake (*Grifola frondosa*) is a saprophytic fungus that commonly grows on fallen trees or stumps of Fagaceae trees such as *Fagus crenata*, *Quercus serrata* and *Quercus crispula* (Mayuzumi & Mizuno, 1997). It forms large heads on oak trees, *Quercus serrata*. It is one of the fungi that attack the core of these trees (Mizuno & Zhuang, 1995).

This paper will cover the aspects of mushroom cultivation technology, with an emphasis on the introduction of Good Agricultural Practices (GAP), which provides a systematic approach to risk management and the improvement of agricultural processes. Special focus will be placed on the identification and management of critical points in production, such as the selection and admission of quality raw materials, pasteurization temperature, incubation and fruit bearing temperatures, hygiene throughout the process, as well as innovative opportunities for sustainable use of the spent substrate.

RESULTS AND DISCUSSION

For the production of mushrooms, the preparation of the substrate is of particular importance (Figure 1). Seasonal agricultural waste can be used as a substrate. This makes cultivation easy and cheap. The rods obtained by pruning the vines and fruit trees, which are abundant in the Tikvesh region, can be used as a substrate (Quimio, 1986; Tong & Chen 1990; Triratana et al., 1991).

In the Tikvesh region, 80% sawdust from deciduous trees, 10% hay, and 10% bran are used for substrate preparation, which are mixed and filled into bags weighing 4-5 kg (Figure 2). Both processes of pasteurization or sterilization can be performed. The pasteurization process is carried out for 1 hour at 80 °C. After pasteurization, the substrate is removed from the barrel and left in a clean room to cool. Sterilization is performed for 30 minutes at 121 °C. After sterilization, they are removed from the autoclave and left in a clean room to cool.

Figure 1. Preparing the substrate for mushrooms (*Source: Kristina Todorova*)

Figure 2. Filling the polypropylene bags with substrate (*Source: Kristina Todorova*)

After the substrate is sterilized, inoculation is carried out. After cooling, the bags are opened and mixed with the mycelium. The mycelium is packed in 4.5L bags, which is enough to inoculate 10 to 12 bags (Figure 3). 200g of mycelium is placed in each bag (Figure 4). Until use, the mycelium is stored in a regular refrigerator at a temperature of 2-4 °C for a maximum of 2 months. Before inoculation, it is taken out to room temperature for 24 hours.

Figure 3. Mycelium (*Source: Kristina Todorova*)

A

B

Figure 4. A-Planting the pasteurized bags, B-Planting the sterilized bags (*Source: Kristina Todorova*)

Then they are taken to a clean room and the inoculated bags are placed on iron racks, where the incubation process begins.

Incubation requires an optimal relative humidity of 62 to 63 % and an optimal room temperature of 23°C, while the optimal substrate temperature should be 25°C. Incubation lasts 18 days. The maturation phase lasts 30-50 days. In the maturation phase, the optimal temperature should be 24°C, the humidity should also be 80-90%, while the light should be 1000 lux.

Once the plastic bags turn white, 2 cm holes are made at a distance of 20 cm using a scalpel. During the fruiting phase, the relative humidity should be 80 to 90% and the CO_2 concentration should be less than 2000 ppm, and it is at this stage that the first harvest begins (Figure 5).

Figure 5. Fruiting of Reishi (Ganoderma lucidum) (*Source: Kristina Todorova*)

In the fruiting phase, it is important to note that Oyster Mushroom (*Pleurotus ostreatus*) is one of the fastest growing crops, yielding a product in one month. While Reishi, Shiitake, Lion's Mane, and Maitake mushrooms take two months.

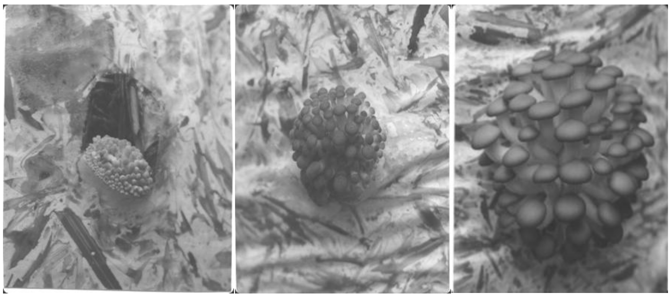

Figure 6. The first beginnings of fruiting of the Bukovka, straw substrate (*Source: Kristina Todorova*)

Harvesting begins before the edge of the mushroom cap straightens and by turning the entire cluster, it is torn from the bag.

Conditions for growing mushrooms

Spots that can be used are garages, basements, abandoned buildings and other places with high humidity. Lighting is an important factor for the proper growth and development of mushrooms, the spot should be well lit. Direct sunlight should be avoided, but mushrooms do not develop in complete darkness. Temperature, the optimal range is around 13-25 °C, at lower temperatures the entire process slows down, and at higher temperatures there is a risk of destruction of the substrate. Humidity should be 80-90%. To ensure this condition, the fruit must be sprayed with water. Ventilation, mushrooms use oxygen when breathing and release carbon dioxide, so it is necessary to be very careful with ventilation. For smaller amounts of substrate, natural ventilation by opening windows and doors (creating a draft) is sufficient. If this factor is not provided, cauliflower-like deformation occurs. Hygiene, the area where they will be grown should be clean and whitewashed.

Problems in mushroom cultivation

Problems with spores in mushroom cultivation

Oyster mushrooms (*Pleurotus sp.*) reproduce sexually by basidiospores located on the lamellae on the underside of the fruiting bodies. When the oyster mushroom matures, that is, when the edge of the fruiting body straightens, it releases spores in huge quantities. If the fruits are not collected in time, the entire room will be covered in spores. Overripe fruits are not of good quality for sale and processing (Figure 7).

Figure 7. Overripe oyster mushrooms (*Pleurotus sp.*) with edges curled upwards, harvesting delayed by 2 days (*Source: Kristina Todorova*)

To avoid spore problems when growing mushrooms, producers should be careful:

- The farm should be as far away from the place of residence as possible,
- Before entering the room, turn on the fan to remove spores from the air,
- Harvest the fruits on time, do not allow the edge of the hat to straighten upwards,
- It is mandatory to wear a protective mask during mushroom harvesting and selection,
- Regularly check exhaust fans, as spores tend to stick to the blades and make engine operation more difficult.

In our population, 10% of people are allergic to oyster mushroom spores. The allergy is characterized by a constant irritating cough, especially intensified upon entering the room where the mushrooms are. If the doctor determines an allergy, the producer should immediately stop growing mushrooms and focus on other production.

Ventilation problems when growing mushrooms

The oyster mushroom is a saprophytic plant that uses plant waste for nutrition, uses oxygen for respiration, and releases carbon dioxide. Therefore, it is necessary to ensure a continuous flow of fresh air in the room where the mushrooms are grown. In unventilated rooms, the concentration of CO_2 is high and can lead to the deformation of the oyster mushroom fruits (Figure 8).

Figure 8. The first signs of deformities in oyster mushrooms are the elongation of the stems, and the mushrooms develop in an inverted form (*Source: Kristina Todorova*)

While amateur mushroom growers typically face minimal ventilation issues due to smaller substrate volumes and adequate ambient oxygen, challenges often emerge when scaling to larger or industrial production, where the pursuit of profit may compromise optimal growth conditions—particularly for rapidly developing oyster mushrooms—necessitating vigilant monitoring, timely ventilation adjustments at the first sign of developmental irregularities, and prompt consultation with experts if conditions do not improve.

Problems with the appearance of insects and spider mites in mushroom production

Mushroom farms are ideal environments for the development of insects and spider mites. They are closely related because the large number of flies helps to disperse the spider mites, which are microscopic in size. It is necessary to exert a large influence because these pests produce a huge number of individuals in a short period of time. Fungus gnats cause damage through their larvae, which eat the mycelium, or cause worming of the fruiting bodies. These are dipterous insects that are divided into several species, namely:

- Sciaridae flies (Sciarid flies: *Lycorilla uripila*, *Lycoriella mali*),
- Phorid flies (Phoridae: *Magaselia nigra*, *Megaselia tamiladuensis*),
- Pinworms or tsetse flies (Cocydomidae: *Heteropeza pygmaca*).
- Spider mites (Histiostomatidae: *Histiostoma* sp.) (Ziberoski & Dimovski, 1993).

Preventive hygiene measures in the production process:

- Maintaining hygiene in the room, proper disinfection in the premises, and proper thermal treatment of the substrate (Ziberoski & Dimovski, 1993).
- Sanitary and hygienic procedures are the main control measures for these pests.
- Returning any waste, and mushroom residues inside or outside the company, there is a risk of transferring pests to the new compost.
- The nets located on the ventilation openings should be smaller than 1mm.
- Thorough sterilization of the substrate. Very important for the control of spider mites.

Protection measures:

The development of mushrooms is monitored from sowing to the final harvest. If the mycelium develops well and quickly at the beginning, then the mushrooms become resistant to many diseases. The problem with midges is very big, especially if they have gained a lot of momentum. Adhesive insect tapes can be used. The best protection against insects is special extermination lanterns. Insects attracted by the light enter the lantern and are killed by the high voltage of electricity from the network placed around the lamp.

Problem with the appearance of green mold (*Trichoderma* sp.) in mushroom cultivation.

These are cellulose fungi, which most often occur in poor-quality substrate and improper heat treatment, during which the spores of the green mold are not destroyed. If an infected substrate is used and if the heat treatment is not performed properly, then these molds are activated during the incubation of the substrate after seeding with mycelium, and especially during fruiting (Ziberoski & Dimovski, 1993, 1993). Protection measures should be taken such as the purchase of quality straw, and sawdust, proper monitoring of the pasteurization or sterilization procedure (technological list), and thorough cleaning and disinfection of the facilities.

The effective implementation of Good Agricultural Practices (GAP) is founded on the systematic identification and mitigation of agricultural risks, reinforced by comprehensive farmer education, clear communication of standards, and integrated awareness campaigns, while annual reviews and continuous procedural updates ensure full traceability, enable rapid problem-solving, enhance long-term efficiency, and support the prompt correction of non-compliance—thereby aligning operations with evolving regulatory standards and best practices to uphold the highest levels of quality, safety, and sustainability in agricultural production.

Critical points in mushroom cultivation technology include:

- Receiving quality raw materials, ensuring that the raw materials used, such as substrate, mycelium are of the highest quality. Regular testing for the presence of contaminants and appropriate documentation for each receipt is required.

- Pasteurization of the substrate: Maintaining the correct temperature and duration for pasteurization is key to destroying pathogenic microorganisms, which can reduce yield or compromise quality.
- Temperature and humidity during incubation, control of these parameters is essential for successful growth of the mycelium in the substrate. Even small deviations can lead to uneven growth or the appearance of contamination.
- Temperature and conditions for fruiting, it is necessary to maintain optimal temperature, light, and air circulation (ventilation) to enable quality and abundant fruiting.
- Hygiene of equipment and space, regular cleaning and disinfection of work surfaces, tools, and equipment reduces the risk of contamination and ensures product safety.
- Traceability and documentation, introducing a monitoring system, which includes record sheets for each stage of production, in order to identify and correct any discrepancies in a timely manner.

The characterization of mushroom cultivation as one of the fastest and most practical agricultural ventures—coupled with the strategic repurposing of abandoned facilities—underscores the economic viability and sustainability of applying Good Agricultural Practices (GAP) in spatially constrained environments, thereby offering a scalable and inclusive model particularly advantageous for resource-limited farmers.

The utilization of spent mushroom compost as an organic manure and soil conditioner in horticulture, or as a dietary supplement in poultry farming, has received some consideration (Rinker, 2002; Santos et al., 2005; Machado, Souza Dias, Santos, & Freitas, 2007; Azevedo, Ávila, Souza Dias, Bertechini, & Schwan, 2009; Ribas et al, 2009). Typically, mushroom farms are generally eager to dispose of compost that is no longer capable of producing viable yields of mushrooms since the residue attracts flies and other insects that can carry diseases, and constitutes a potential source of water and air pollution (obnoxious odors) (Zied et al., 2011). On the other hand, spent compost from the cultivation of Agaricus spp. represents a possible alternative substrate for the production of lettuce since the material exhibits some characteristics that are appropriate for the growth of seedlings (Uzun, 2004; Medina et al., 2009; Ribas et al., 2009; Fidanza et al., 2010; Zhang et al., 2012). Substrate comprising a mixture of 45% spent mushroom compost from *A. subrufescens* provided the most adequate conditions for the growth and development of lettuce seedlings and, consequently, of vigorous marketable plants. The data confirmed that superior quality lettuce seedlings give rise to high quality marketable heads, and even the treatment with the highest amount of SMS (75%) resulted in seedlings of higher quality compared to commercial substrate. *Agaricus subrufescens* SMS is, therefore, an excellent substrate component for producing lettuce seedlings of high quality (Marques et al., 2014).

About 1 kg of mushroom production will produce 5 kg of spent residual material called spent mushroom substratum (SMS) (Lau et al. 2003). An ordinary farm disposes of more than 24 t of SMS per month. After the final crop harvest, the spent mushroom substratum (SMS) still has several positive attributes left for its promising utilization (Singh et al. 2011). The material has been discovered to be a rich source of nutrients for horticultural crops. Along with this, it exhibits immense cation exchangeability and has a low mineralization rate that maintains its quality as an organic matter. Mushrooms through bioconversion processes reutilize the spent substrates and convert the polluting material into valuable food products (Beyer, 2005; Noble, 2005). They can accumulate and filter pollutants and act as one of the most promising and effective bio sorbents of toxic heavy metals from radioactive nuclear fallout polluting water and soil, thereby acting as neutralizers of pollutants (Das, 2005). Mushroom farming is the most efficient way of agro-industrial disposal and converting these residues into the mushroom substrate to grow mushrooms and its spent mushroom substrate has various applications to make it a zero-emission strategy for food production (Niazi & Ghafoor, 2021).

Integrated mushroom cultivation exemplifies a sustainable agricultural strategy by converting agricultural waste—such as crop residues, straw, and sawdust—into valuable mushroom biomass, thereby enhancing resource efficiency, generating additional income for farmers, reducing environmental impact through decreased waste production, and contributing to improved soil health and nutrient cycling, as supported by recent studies (Kushwaha, 2023).

According to Bae et al. (2006), chemical composition of spent mushroom substrates from saw dust was natural detergent fiber 78.2%, acid detergent fiber 60.4%, hemicellulose 17.8%, cellulose 40.4%, lignin 20.0%, nonfibrous carbohydrate 7.8%, crude protein 7.2%, true protein/crude protein 69.4%, non-protein nitrogen/crude protein 30.6%, acid detergent fiber-crude/crude protein 36.4%, ether extract 2.1%, crude ash 4.7%, and dry matter 40.8%. The results reveal that spent mushroom substrate from sawdust based is suitable in vermicomposting and with the specific ratio, it not only can accelerate the mineralization of nutrients but also proved as a better feed material for vermiculture. Thus, the generation of organic waste from the agriculture sector indirectly can be reduced or eliminated, especially in mushroom cultivation sectors (Nik Nor Izyan et al., 2009).

Spent mushroom compost (SMC) is rich in salts, organic material, enzymes, and nutrients, making it a suitable habitat for microbes like bacteria and fungi, which aid in disease suppression and plant growth (Patil et al., 2018). SMC's disease-suppressive properties depend on microbial activity, nutrient content, and chemical and physical factors. It serves as a biocontrol agent against plant diseases due to its organic salts, enzymes, and antagonistic microbes (e.g., *Trichoderma sp*.). SMC reduces the need for fungicides and fertilizers, lowering costs, improving soil health, and aiding in soil remediation. Spent mushroom substrate (SMS) refers to non-composted residues after mushroom harvest, while SMC refers to composted residues, each varying in nutrient content for biofertilizer use (Singh et al., 2020).

CONCLUSIONS

The introduction of Good Agricultural Practices (GAP) in mushroom cultivation ensures traceability, transparency, and safety across the production cycle, thereby enhancing product quality, reinforcing consumer trust, promoting environmental stewardship, and aligning with regulatory standards while advancing food safety, ecosystem preservation, and the protection of both workers and consumers—ultimately strengthening producer credibility and fostering sustainable agricultural practices. For successful production in mushroom cultivation, the following is required: proper sterilization, the sterilized bags should be cooled to 20-25 °C and then inoculated the same day, the space where they will be grown should be completely clean and whitewashed, and special attention should be paid to ventilation, which is why it is necessary to ventilate the room and maintain a relative humidity of 80 to 90%.

The implementation of GAP in mushroom farming not only improves the safety and quality of products, but also creates a management system that facilitates the monitoring, adaptation and improvement of agricultural practices. This contributes to the sustainability and competitiveness of the industry.

The integration of mushroom cultivation into agricultural systems exemplifies a zero-emission, sustainable approach to resource optimization by transforming agricultural waste into high-value substrates, with spent mushroom substratum (SMS) and compost (SMC) offering multifaceted benefits—including enhanced soil fertility, nutrient cycling, disease suppression, and microbial enrichment—while serving as versatile post-harvest resources for vermicomposting, soil conditioning, firewood, and horticultural substrates, thereby reducing reliance on chemical fertilizers, remediating environmental contaminants, and advancing ecological balance in sustainable farming systems.

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VALORIZATION OF ONION SKIN WASTE FOR BIOACTIVE COMPOUNDS

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ABSTRACT

Onion (*Allium cepa L.*) is among the most widely cultivated and consumed vegetables globally, generating significant biowaste. About 37% of fresh onions are discarded during industrial processing, primarily as onion skins and peels. These skins are a valuable agricultural by-product rich in bioactive compounds, making them beneficial in various sectors, including agriculture, the food industry, and bioplastics. They contain high levels of flavonoids and antioxidants, particularly quercetin, which can enhance soil health when composted or used as organic fertilizers, thereby promoting sustainable agricultural practices. This study aims to optimize extraction methods for bioactive compounds from onion skin and evaluate their potential applications as food, agricultural and cosmetic products. Various extraction techniques were employed to identify the most effective method for extracting bioactive compounds using solvents such as ethanol, methanol, and water. Ultrasound-assisted extraction was utilized to enhance the release of these compounds and compare its efficiency with traditional methods like maceration and Soxhlet extraction. The FTIR analysis of onion skin and its extracts demonstrated the presence of various bioactive compounds, such as phenolics, flavonoids, lipids, carbohydrates, and amino acids, highlighting the rich chemical composition of these materials. The findings provide a comprehensive understanding of the multifaceted applications of onion skins, emphasizing their role as a sustainable resource rich in bioactive compounds with potential benefits across health, agriculture, and industry. By repurposing onion skin in these applications, agricultural waste can be minimized, contributing to a more sustainable and circular economy.

Key words: *Biowaste, Onion skin, Extraction, Characterization*

INTRODUCTION

Biowaste presents a significant global challenge, with negative environmental and economic impacts. This issue is particularly pronounced in the agricultural sector, where large quantities of organic waste are generated. Onion (*Allium cepa L.*) is among the most widely cultivated and consumed vegetables globally, ranking as the second-most important horticultural crop after tomatoes (Celano et al., 2021). The annual production of onions exceeds 60 million tons. This significant volume of production correlates with a substantial amount of waste generated during processing, primarily in the form of onion skins and peels. In the European Union, annual onion waste generation is reported to be as high as 500,000 tons (Zhang et al., 2024; Črnivec et al., 2021; Celano et al., 2021).

The disposal of onion waste poses environmental challenges, as traditional methods such as landfilling can contribute to greenhouse gas emissions (Črnivec I.G.O., et al., 2021;

Segundo R.F., et al., 2022). On the other hand, research indicates that onion skins are rich in bioactive compounds that can be validated for various applications. They contain beneficial compounds that can be extracted and utilized in various industries (Celano R., et al., 2021).

Onion waste contains around 8.3–15.6% protein, 88.56% carbohydrates, and 169–750 mg/g of dietary fiber, making it a valuable source of nutrients. Because of this rich composition, these compounds are being explored for their potential use in functional foods. (Shabir, I. et.al., 2022). Incorporating powdered onion waste into various food products can enhance their nutritional profile by adding fiber, antioxidants, and other beneficial compounds. Despite this, it can optimize the taste and texture of food products. Onion skins are a concentrated source of flavonoids, especially quercetin, which is known for its powerful antioxidant properties. Antioxidants help combat oxidative stress in the body, potentially reducing the risk of chronic diseases (Zhang, Q., et al., 2024).

The antioxidant properties of onion skins are also being explored for their potential benefits in skin care products. Bioactive compounds such as quercetin exhibit strong antioxidant activity, which may help protect the skin from oxidative stress and aging (Messias, M. et al., 2023).

Additionally, composting onion waste with other organic materials can produce high-quality compost that improves soil fertility and structure while reducing the need for synthetic fertilizers. Pellejero (2015) research showed that these mixtures produced compost with significant agronomic value, achieving efficiencies of nearly 50% for the best combinations. This compost can be used as a valuable organic fertilizer, enhancing soil quality and supporting sustainable farming practices. The study emphasizes the importance of using agricultural byproducts to reduce environmental impact and improve soil fertility and crop production.

Innovative extraction methods have been studied to effectively recover these bioactive compounds. The valorization of onion skin waste aligns with current trends in sustainable agriculture, food, and cosmetic production, highlighting the importance of converting waste into valuable resources. By transforming onion waste into high-value products, we can not only reduce environmental impacts but also improve food quality and safety.

MATERIALS AND METHODS

The collected skin from red onion (*Allium cepa* L.) was used as starting material. It was washed using distilled water to remove dirt and other impurities and left to dry in an open container. Afterwards, the dried peels were placed in oven drier at 70°C for 5 h and grounded to obtain fine powder using electric mill. The powder thus obtained was stored in food grade polythene packages free from environmental climatic changes, till usage.

Extraction Procedures

Maceration

For maceration extraction (MAE) of onion skin, three solvents (methanol, ethanol and water) were utilized to maximize the extraction of bioactive compounds. Onion powder, prepared by drying and grinding the skins, was weighed accurately and placed in covered beakers. The samples were soaked in their respective solvents at room temperature (*25°C*) for duration of 30 hours. Periodic stirring was applied to enhance solvent penetration and ensure uniform extraction. After the extraction period, the mixtures were filtered using Whatman filter paper (No. 1) to separate the liquid extract from solid residues. The resulting filtrates were collected for subsequent analyses, such as spectroscopic evaluation.

Soxhlet extraction

The *Soxhlet* extraction process was initiated by carefully packing 3 g of finely shredded onion skin into a thimble, which served as the solid sample holder. This setup was placed inside the extraction chamber of the *Soxhlet* apparatus. The solvent used for the extraction was 150 mL of 96% ethanol, chosen for its efficacy in extracting bioactive compounds from onion skins.

The extraction was conducted in a continuous cycle over a period of 18 hours. During the process, the ethanol was heated in a round-bottom flask connected to the *Soxhlet* apparatus, causing it to vaporize. The vaporized ethanol traveled through the apparatus and condensed in the condenser unit, dripping onto the onion skin within the thimble. The solvent gradually dissolved soluble compounds from the sample and, when the chamber filled to the siphon point, the enriched ethanol returned to the flask. This cycle was repeated multiple times, ensuring efficient extraction of the target compounds.

The prolonged duration of 18 hours allowed the solvent to interact thoroughly with the onion skin, maximizing the yield of extractable bioactive compounds. The final product, a concentrated ethanolic extract, was collected in the round-bottom flask for further processing and analysis.

Ultrasound assisted extraction (UAE)

The Ultrasound Assisted Extraction (UAE) from onion skin was performed using an ultrasonic bath operating at a 40 kHz sonication frequency. The extraction was carried out at a controlled temperature of 64°C for 30 minutes. The ultrasonic bath had a 15 L capacity and was equipped with a 600 W heater to maintain the desired temperature during the process. After sonication, the mixtures were filtered to separate the liquid extract from the solid residues. The extracts were then processed for subsequent analysis to determine the bioactive components.

ATR-FTIR Spectroscopic Analysis

Fourier Transform Infrared (FTIR) spectroscopy with Attenuated Total Reflectance (ATR) was employed to identify the functional groups present in compounds extracted from onion skin. This technique provides valuable information on the molecular structure and chemical bonds in the sample by detecting characteristic frequencies in the infrared spectrum. A small quantity of onion skin extract was directly placed on the spectrometer equipped with the Shimadzu IR Spirit Fourier Transform Infrared Spectrophotometer and a QATR-S Single Reflection ATR accessory. Spectral data were recorded over the range of 4000 to 400 cm^{-1} with 20 scans at a resolution of 4 cm^{-1}. The air spectrum was used as the background. The resulting FTIR spectra were analyzed using LabSolutions IR software to identify peaks corresponding to functional groups such as hydroxyl (-OH), carbonyl (C=O), and aromatic rings, commonly found in polyphenols, flavonoids, and other bioactive compounds in onion skin. These peaks were further compared with published data to confirm the chemical composition and validate the presence of targeted bioactive compounds.

RESULTS AND DISSCUSION

Functional Groups Characteristics

The FTIR spectroscopic analysis of onion skin before extraction was conducted to identify the functional groups present in the raw material. This analysis provides baseline data on the molecular composition of the onion skin, which is rich in bioactive compounds like flavonoids, phenolics and lipids. The spectral characteristics of onion were shown in Fig. 1. Key peaks identified in the FTIR spectrum included: Peaks around 2928 cm^{-1} representing C-H stretch that is due to CH_2 asymmetric stretch of methyl groups mainly from lipids (Lu et al., 2011) and 2849 cm^{-1} stretching vibrations for – CH_2 group (Adu et al. 2022). The small peak around 1748 suggests the presence of carbonyl group (Verma *et al.* 2018).

Sharp peaks near 1580-1650 cm^{-1} are assigned to ring C-C stretch of phenyl (Lu et al., 2011), which is present at high levels in the polyphenolic components in *Allium* plants. The peak that appeared at 1454 cm^{-1} confirmed the presence of C–N groups for the chlorophyll group. Aromatic group C=C was detected by the small peak at wave numbers of 1502 cm^{-1} (Adu et al. 2022). The band at 1405 cm^{-1} is due to CH_3 asymmetric deformation. The peak at 1339 cm^{-1} is due to the in-plane C-O stretching vibration combined with the ring stretch of phenyl (Lu et al., 2011). The minor band at 1255 cm^{-1} is from amide III for protein (Lu et al., 2011).

The wavenumber region between 1200 and 950 cm^{-1} contains functional groups mainly from carbohydrate. The ‘‘shoulder’’ peak at 1100 cm^{-1} is from carbohydrates while the bands at

1015 and 955 cm^{-1} are due to vibrational frequency of -CH_2OH groups of carbohydrates and OCH_3 from polysaccharides-cellulose (Lu et al., 2011).

These findings serve as a reference for comparing chemical changes after extraction, enabling the evaluation of specific compounds removed during the extraction process.

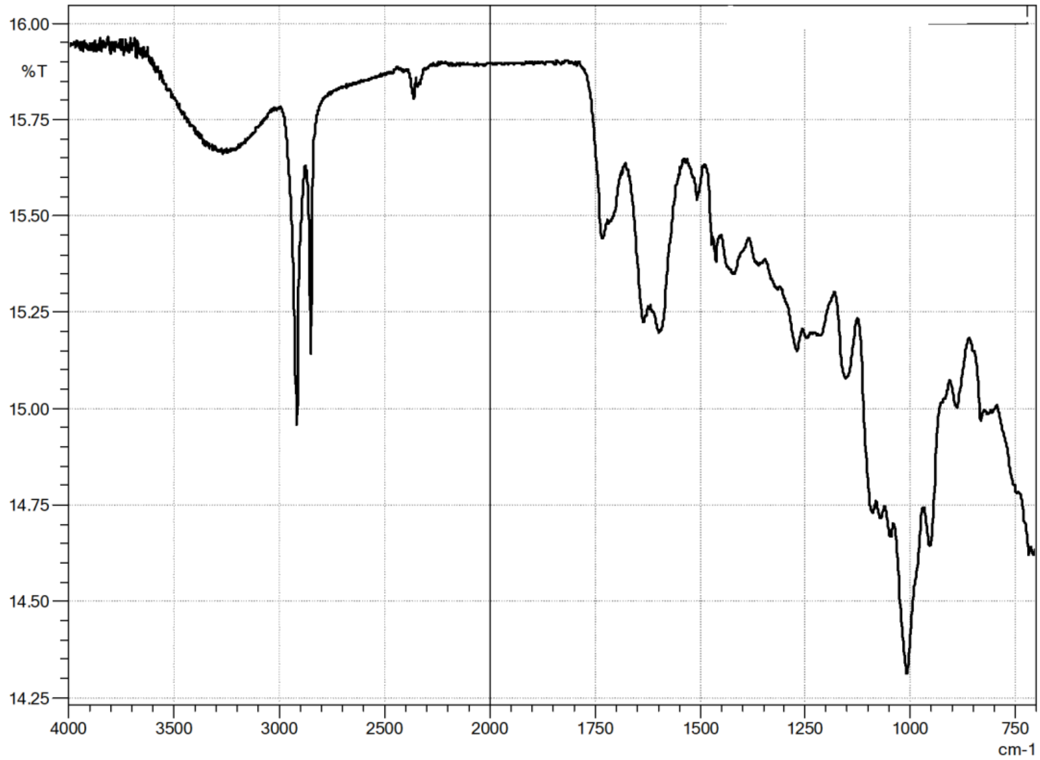
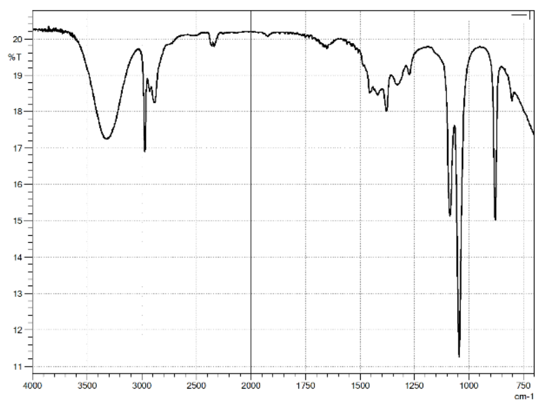
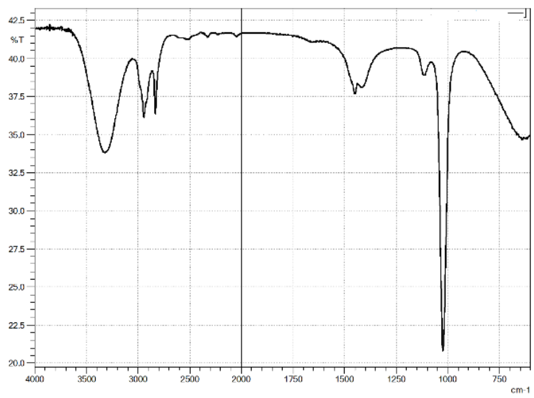
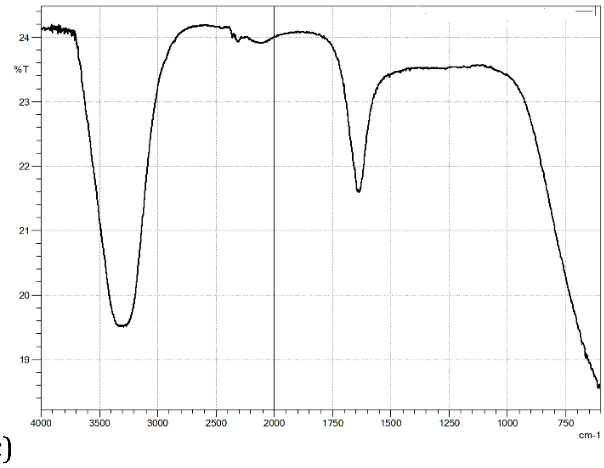


Figure 1. FTIR analysis of onion skin powder before extraction.

The FTIR spectra obtained from the onion skin extract, prepared using maceration extraction, are presented in Figure 2. The spectra revealed several distinctive peaks at various wavenumbers, indicating the presence of multiple functional groups characteristic of bioactive compounds in the extract. The bands between wavenumbers 1800-750 cm-1, (fingerprint regions) reflected biochemical compounds, especially parts of the secondary structures of carbohydrates, lipids, proteins, and polyphenols in the plant.

a) 

b) 



c)

Figure 2. FTIR analysis of the extract from onion skin extracted with maceration (solvents: a) ethanol, b) methanol and c) water)

Peaks in the region of 3200–3300 cm^{-1}, corresponding to hydroxyl (-OH) stretching vibrations, suggesting the presence of phenolic compounds. Peaks near 2800–3000 cm^{-1}, attributed to C-H stretching from aliphatic and aromatic structures. Small peaks were observed around 1300-1450 cm^{-1} suggesting the presence of alkane from methyl group (Ateeq et al., 2023).

A strong band in the 1000–1200 cm^{-1} range, associated with C-O stretching, is commonly found in alcohols, ethers, or carbohydrates (Adu and Gelyaman, 2023). The medium peak around 870 cm^{-1} indicates the presence of bending vibrations of aromatic C-H bonds (Egbujor, *et al.* (2023)

These spectral features confirm the presence of diverse functional groups in the onion skin extract, reflecting its rich composition of phenolics, flavonoids, and other bioactive compounds. This analysis serves as a foundation for further chemical characterization and potential application of the extract.

The FTIR analysis of the ethanol extract from onion skin prepared using *Soxhlet* extraction revealed a spectrum almost identical to that of the ethanol extract obtained with maceration. Both methods produced similar spectral features, indicating the presence of common functional groups such as hydroxyl (-OH) groups, C-H stretching vibrations, and aromatic and carbohydrate structures, which are characteristic of bioactive compounds.

The only notable difference lies in the intensity of certain peaks, which may reflect slight variations in the concentration of bioactive compounds due to differences in extraction efficiency between the two methods. These findings suggest that both *Soxhlet* and maceration methods effectively extract the key bioactive components from onion skin, with minor quantitative differences.

The IR spectrum of onion extract extracted with Ultrasound assisted extraction showed a prominent peak around 3200-3400 cm^{-1}, corresponding to the stretching vibration of the O-H group in phenols. One small peak was observed at 1412 cm^{-1}, which can be attributed to the O-H bending vibration characteristic of carboxylic acids. These peaks indicate the presence of functional groups commonly associated with phenolic and carboxylic acid compounds in the onion extract (Egbujor, et al. 2023). Aligning with the findings of Rodríguez Galdón et al., (2008), the observed peaks confirm the presence of carboxylic acids in the onion extract. The C–H stretching vibrations are observed at wavenumbers 2854 cm^{-1} and 2924 cm^{-1}, corresponding to the stretching of the C–H bonds in methyl (−CH3) and methylene (−CH2) groups, respectively (Adu and Gelyaman, 2023). Their study identified carboxylic acids as a major organic acid group in onions, with significant proportions of citric acid (48.5%), malic acid (43.6%), tartaric acid (18.8%), and oxalic acid (11.3%).

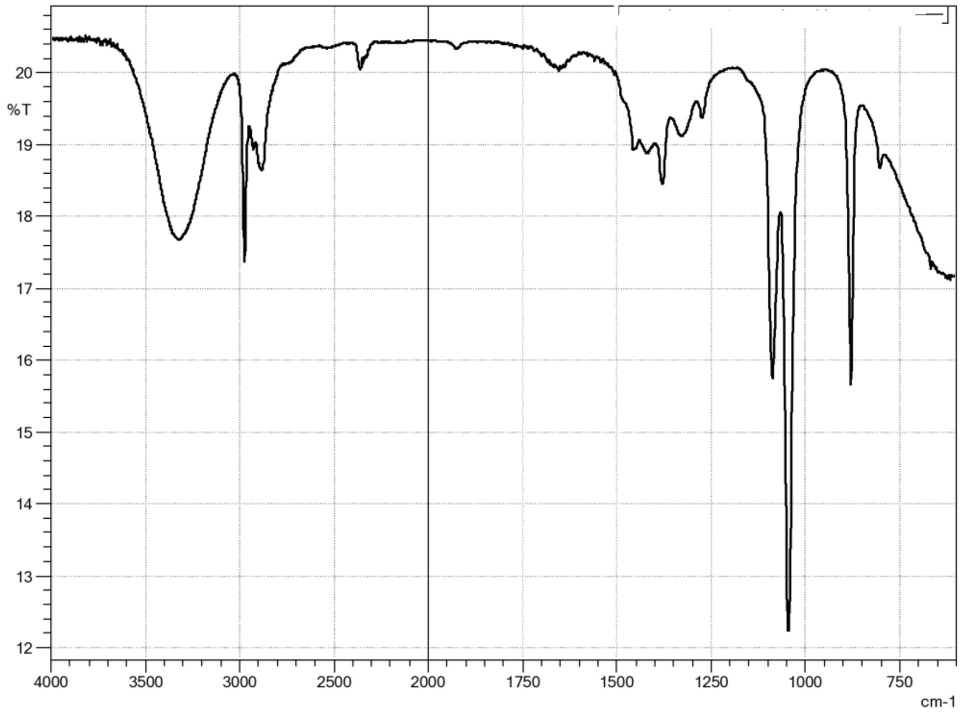
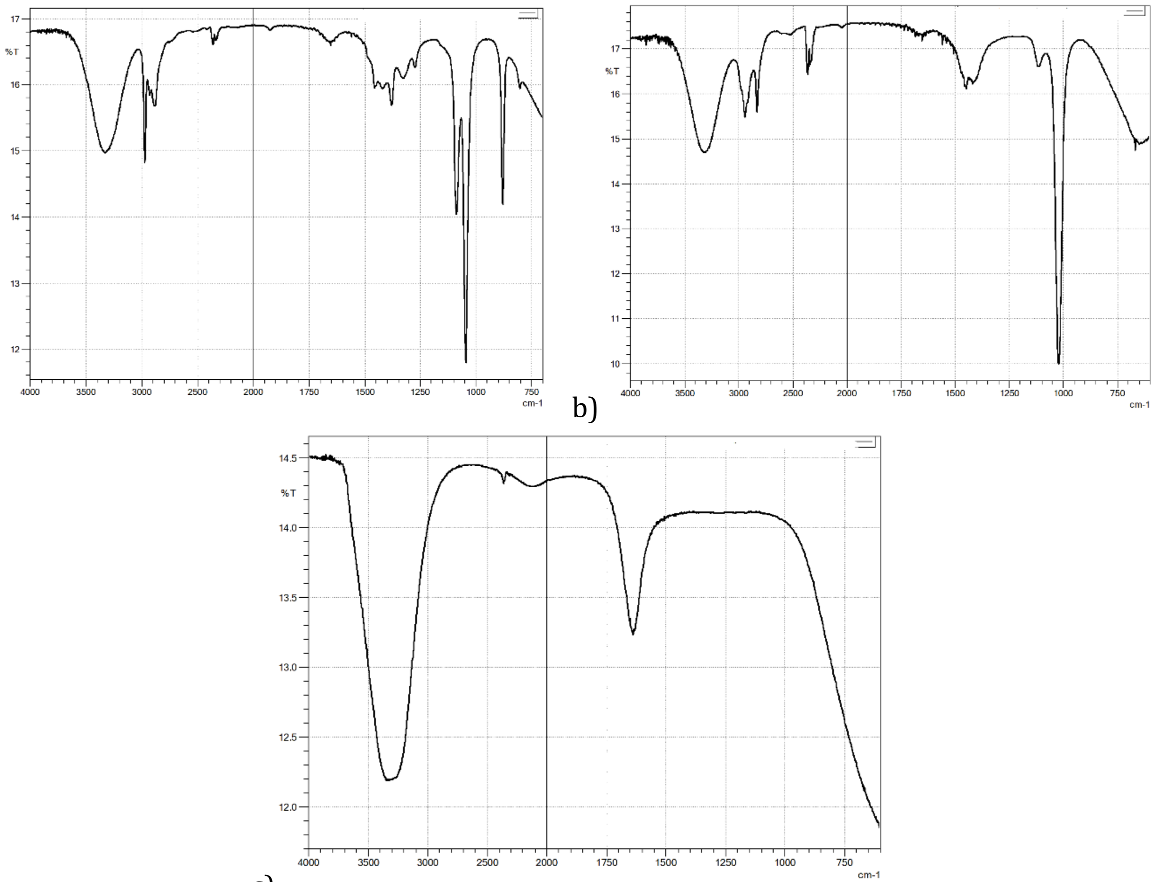


Figure 3. The FTIR analysis of the ethanol extract from onion skin, prepared using *Soxhlet* extraction

The peak around 1600 cm^{-1} in the FTIR spectrum of a water extract from onion skin suggests the presence of C=C stretching vibrations from aromatic rings or C=O stretching vibrations. This peak indicates the presence of bioactive compounds like phenolics, or aromatic structures emphasizing the extract's functional and antioxidant potential (Adu and Gelyaman, 2023). The small peak around 2880 cm^{-1} corresponds to the stretching vibration of the N-H bond in amino groups, which suggests the presence of amino acids in onions. Amino acids in onions are good for health, supporting detoxification, antioxidant activity, and overall wellness. The peak at 1355 cm^{-1} is attributed to the in-plane bending vibration of the O-H bond, indicating the presence of hydroxyl groups (Wang et al., 2022). The peak at 1255 cm^{-1} corresponds to the C-O stretching vibration of carboxylic acids, while the peak at 1050 cm^{-1} is attributed to the C-O stretching vibration of alcohols. Additionally, the absorption band at 870 cm^{-1} corresponds to the stretching vibration of the C-H bond, indicative of aromatic structures presents in onions. These findings align with the high phenolic content reported in onions (Egbujor, et al. (2023).

According to Swer et al. (2018), the presence of O–H, C=O, C=C, and C–O–C functional groups in the FT-IR spectra is characteristic of anthocyanin compounds. These features are essential for identifying and characterizing anthocyanin compounds in a sample.

The spectral features of all onion extracts displayed a high degree of similarity, indicating the presence of common functional groups and bioactive compounds across the samples. However, variations were observed in the intensities of the peaks, which suggest differences in the concentration or abundance of these bioactive compounds in each extract. Such variations may arise from differences in the extraction methods, solvents used, or specific conditions applied during the extraction process, which can influence the efficiency of compound recovery.



a) b) c)

Figure 4. FTIR analysis of the extracts from onion skin extracted with Ultrasound assisted extraction (solvents: a) ethanol, b) methanol and c) water)

These intensity variations provide valuable insights into the effectiveness of extraction techniques and the relative composition of bioactive substances in the extracts. Table 1 includes data regarding Fourier Transform Infrared Spectroscopic (FTIR) analysis of the obtained onion skin powder and extracts which was done to analyze the presence of functional groups.

Table 1: FTIR analysis of onion skin powder and its extracts from different methods

No.	Peak ranges (cm^{-1})	Functional groups
1.	3300-3200	Hydroxyl group (H-bonded–OH- stretch)
2.	3000-2800	attributed to C-H asymmetric stretch of methyl groups
3.	1750-1700	C=O stretch / carbonyl group
4.	1650-1500	C=C stretching vibrations from aromatic rings C=O stretching vibrations C-C / phenyl
5.	1450-1300	-C-O stretching/ vibration in plane/ phenyl CH3 asymmetric deformation O-H bend/ Alcohol/ carboxylic acids
6.	1300-1100	C-O stretch / Carboxylic acids C-N / amide III for protein -C-C- stretch, -C-O stretch / ethers, primary alcohols
7.	900-800	Skeletal –C-H- vibrations

CONCLUSION

Onion skin, a significant component of onion waste, poses environmental challenges due to its strong aroma and limited use as animal fodder. However, it is a rich source of bioactive compounds, particularly phenolic compounds, with potential applications in food, pharmaceuticals, and cosmetics. These bioactive compounds, especially flavonoids like quercetin, are known for their antioxidant, anti-inflammatory, and antimicrobial properties. Valorizing onion skin can help mitigate its environmental impact by reducing waste and promoting sustainability, while also providing natural antioxidants for food preservation. Additionally, onion skin can serve as a functional ingredient to enhance the nutritional and health benefits of various products, offering a natural alternative to synthetic additives. By unlocking the potential of this agricultural byproduct, we can create eco-friendly solutions that contribute to a circular economy and promote overall well-being.

The FTIR analyses of onion skin and its extracts revealed the presence of diverse bioactive compounds, including phenolics, flavonoids, lipids, carbohydrates, and amino acids. Key functional groups such as hydroxyl (O-H), carbonyl (C=O), and C-H stretching in methyl and methylene groups were identified, along with aromatic and polysaccharide structures. Distinct spectral features in the extracts highlight the rich chemical composition of onion extracts, providing baseline data for evaluating chemical changes and potential applications.

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SECTION 3

PLANT PROTECTION

UDK: 636.649-152.61:632.38(497.7)

DETERMINING THE VIRUS STATUS OF SOME MACEDONIAN AUTOCHTHONOUS EMBROIDERED PEPPER LANDRACES

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ABSTRACT

The Institute of Agriculture is holder of the Macedonian National Gen Bank, where the seedling material of many autochthonous, newly created, introduced and high potential varieties and populations of cereals, vegetable, fodder and industrial crops are preserved. All these plant genetic materials represent the basis for biodiversity conservation, starting material in selection programs for creating new varieties and for examination and evaluation of the agro-biological and technological properties of the introduced and already established varieties and populations. That is why, it is of crucial importance that this material is in good condition, especially the health status of the conserved seeds, since some of the diseases can be seed-transmitted. Because the collected plant material in the Gen Bank had never been tested for seed-transmissible pathogens, as a pioneer examination, 13 autochthonous embroidered pepper landraces from the National Gen Bank were selected and tested for two viruses: Alfalfa Mosaic Virus (AMV) and Tobacco Mosaic Virus (TMV). Virus detection was conducted with the use of the DAS-ELISA method. After the evaluation, it was determined that AMV was the most prevalent virus. While TMV infection was observed in only few of the tested samples, in most of the examined material AMV was detected. The infected seeds were excluded from further regeneration. This type of investigation should be continued on other crops, in order to determine virus-free material that will be used for further regeneration.

***Key words**: Alfalfa mosaic virus, Tobacco mosaic virus, seed-transmission, autochthonous pepper landraces.*

INTRODUCTION

The National Gen Bank for Plant Material is a place where the seedling material of many autochthonous, newly created, introduced and high potential varieties and populations of cereals, vegetable, fodder and industrial crops are preserved. In some countries, the National Gen Bank works as a separate institution, while in others is part of already functioning Scientific Institutions. The Institute of Agriculture – Skopje is holder of the Macedonian National Gen Bank for preserving the plant material of autochthonous, newly created and introduced high potential varieties of agricultural crops. The importance of maintaining the plant genetic material lies in biodiversity conservation, representing basis in plant variety selection programs and in examination and evaluation of the agro-biological and technological properties of the introduced and already established varieties and populations.

One of the richest collections in the Macedonian National Gen Bank is the pepper collection, consisting of various pepper varieties and landraces, among which are the autochthonous embroidered pepper landraces, characteristic for this region. Because it is of great national importance to preserve this collection, regeneration of the collected seedling material is of great importance. In order to produce healthy genetic material, the stored pepper samples should be tested for seedborne diseases. As one of the most important disease casual agents associated with pepper production in R. N. Macedonia are virus infections (Jovancev et al., 1996; Rusevski et al., 2011; 2013). Most important seedborne virus infections found on peppers are caused by: Alfalfa mosaic Virus (AMV) (Shutich, 1995; Janecek, 2023) and Tobacco Mosaic Virus (TMV) (Demski, 1981; Shutich, 1995). These viruses were previously detected in the Macedonian pepper production in varoius regions (Rusevski et al., 2011; 2013), so their presence in the collected samples can be expected.

Because the collected plant material in the Macedonian Gen Bank had never been tested for seed-transmissible pathogens before, as a pioneer examination we have tested the seed collection of embroidered pepper landraces for these two economically important seedborne viruses (AMV and TMV).

MATERIALS AND METHODS

For testing for seed-transmissible viruses, 13 autochthonous embroidered pepper landraces from the National Gen Bank were selected. They had the following accession numbers: 1591, 955, 975, 993, 986, 956, 1727, 1750, 1598, 16990, 1700, 1592 and 976 (Figure 1). The selected seedling material was cultivated during the vegetation of 2023 on the experimental plot of the Institute of Agriculture-Skopje in Kochani.

From the grown pepper plants, field inspection was conducted along a transect and sampling of ten randomly chosen plants per landrace was performed. Leaves from the uppermost 2–3 branches of each plant were collected. The collected leaf samples were tested for two pepper seed-borne viruses: Alfalfa Mosaic Virus (AMV) and Tobacco Mosaic Virus (TMV) with the use of Double antibody sandwich ELISA (DAS-ELISA) method using commercial kits (Sediag, France), as described by Clark and Adams (1977) and modified as proposed by the manufacturer (Figure 2). Plant tissue samples were homogenized in an extraction buffer (1:10 w/v). Positive and negative controls produced by the same manufacturer were included in each plate. Samples were considered positive if the average optical density (OD) after one hour incubation at temperature of 37^{o}C in the dark was higher than twice the average OD of the negative control, measured with an ELISA microplate reader Multiskan FC (Thermo Scientific, USA) at absorbance of 405 nm.

Figure 1. Fruit samples of some of the investigated embroidered pepper landraces

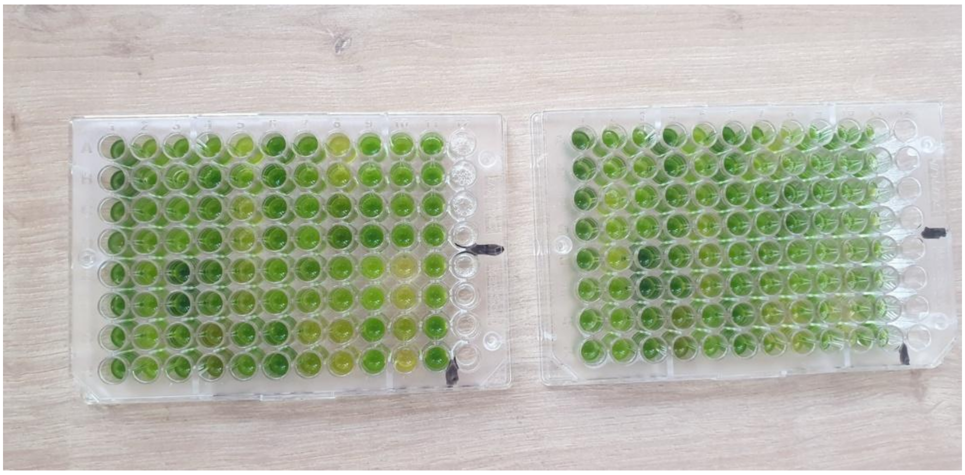

Figure 2. Testing of Macedonian pepper plant landraces from the National Gen Bank with the use of the DAS-ELISA method

RESULTS AND DISCUSSION

After the evaluation of the Macedonian autochthonous embroidered pepper landraces, it was determined that AMV was the most prevalent virus. AMV was present in 10 of the 13 tested landraces, with a percent of infection in some landraces as high as 90% (1591 and 975). TMV infection was observed in only 2 of the tested landraces (993 and 986). In the landrace no. 993, TMV was observed in single and in mixed infections, while in 986, TMV was observed only in mixed infections with AMV.

Due to the very high infection percentage of AMV among the tested samples, in order to exclude any contamination of the tested material, the DAS-ELISA test was repeated. After the test repetition, we have obtained the same results, confirming the high percentage of AMV infection among the tested pepper plants. The seeds from the infected pepper plants were excluded from further conservation in the National Gen Bank collection.

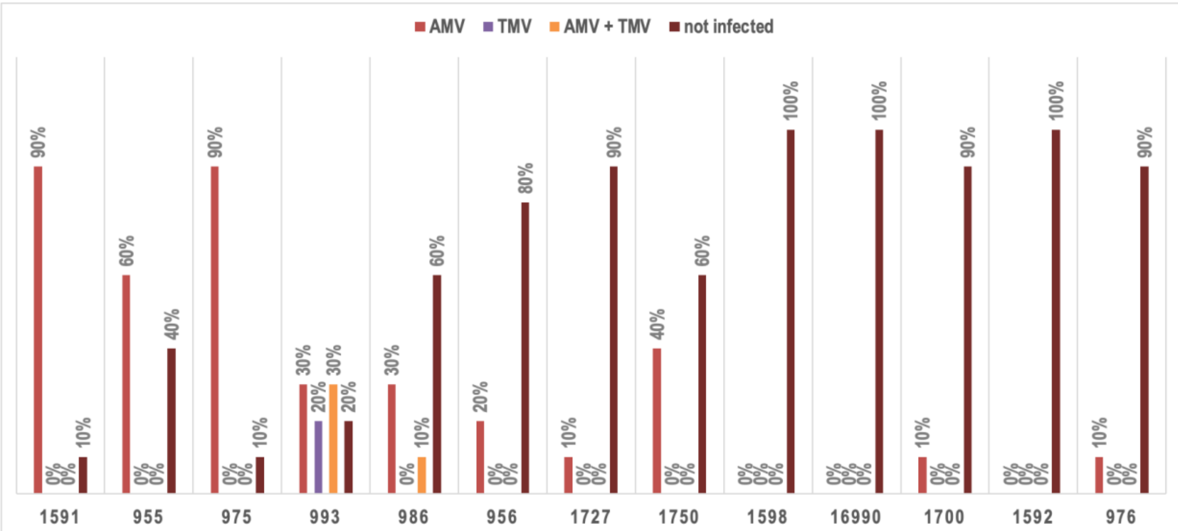

Figure 3. Virus status of embroidered Macedonian pepper landraces from the National Gen Bank for pepper seed-borne viruses in 2023 (% of infection)

This kind of prevalence of AMV is not usual in the Macedonian pepper production (Rusevski et al., 2011; 2013; Bandjo Oreshkovikj et al., 2018), as well as in other pepper production regions in the world (Ormeño et al., 2006; Miloshevich, 2013). Still, it was observed in our study and confirmed by serological tests. The explanation may be that during the years of regeneration of the embroidered pepper genetic material, accumulation of the virus occurred through the years, since no health control had been previously applied to the collected material, apart from visual inspection.

Seed transmission of viruses may cause systemic infections after germination, exposing plants at their youngest and most vulnerable stage (Kaur et al. 2020). Infected seeds are the primary source of viral pathogens in commercial production, and unlike bacterial or fungal pathogens, no effective seed treatments for viruses are currently available (Kaur et al. 2020; Sastry 2013).

CONCLUSION

This is a pioneer research provided on part of the collected seedling material from the Macedonian National Gen Bank. Since the obtained results in our study showed the importance of health monitoring of the preserved collections, especially for seedborne viruses, we recommend that this type of investigation continues in the future on other plant genetic material stored in the Gen Bank.

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UDK: 632.937.1:579.852.11]: 632.952(497.784)

ANTIFUNGAL ACTIVITY OF SOIL BACTERIAL STRAINS FROM THE GENUS *BACILLUS* ISOLATED FROM CONTAMINATED SOIL FROM THE BITOLA REGION (REK)

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ABSTRACT

In recent years, there has been a growing focus on developing bio-based products, with species from the *Bacillus* genus frequently utilized due to their high production of antimicrobial compounds and their resilience to harsh environmental conditions through endospore formation. In this context, the antifungal potential of bacterial isolates from contaminated soil in the Bitola region, with a focus on the genus *Bacillus* was investigated. The isolates were tested against several phytopathogens, including *Plasmopara viticola*, *Erysiphe* sp., *Peronospora*, *Alternaria alternata*, *Aspergillus ochraceus*, *Penicillium* sp. and *Monilinia fructigena*. The methodology involved bacterial isolation from soil samples, followed by antifungal activity assays utilizing the agar well-diffusion method. The findings revealed that *Bacillus* isolates exhibited considerable antifungal activity across all tested pathogens, with the most pronounced effects against *Alternaria alternata* and *Erysiphe* sp. Efficacy varied between intracellular and extracellular fractions, with intracellular fractions demonstrating stronger activity. This suggests that *Bacillus* isolates possess antifungal metabolites with potential applications as natural pesticides. The results suggest potential for developing alternative methods to control phytopathogens, particularly given the increasing resistance to synthetic fungicides. These findings highlight the potential of bacterial agents as sustainable antifungal resources, contributing to eco-friendly agricultural practices within and beyond the Bitola region. Further research is necessary to identify and characterize specific *Bacillus*-derived metabolites to support innovative, environmentally sustainable plant protection strategies.

Key words*: antifungal activity, soil microorganisms, Bacillus, plant pathogenic fungi, secondary metabolites*

INTRODUCTION

Soil represents a fundamental natural resource with crucial roles in ecosystem functioning, including plant growth promotion, nutrient cycling, water cycle regulation, and carbon storage (Doran & Zeiss, 2000). It is a complex and dynamic system composed of minerals, organic matter, water, air, and diverse microbial communities, all of which contribute to biodiversity maintenance and ecological balance. Among soil microorganisms, bacteria from the genus *Bacillus* are particularly significant due to their involvement in nutrient cycling and their capacity for biological control of plant pathogens. However, soil contamination, often resulting from anthropogenic activities such as industrial production, pesticide and heavy metal application in agriculture, inadequate waste management, and urbanization, can significantly alter its microbiological composition and functionality (Alloway, 2013). Such contamination contributes to soil degradation, reduces fertility, and negatively impacts biological activity, particularly affecting beneficial microorganisms and their ability to perform essential agroecosystem functions (Bissett *et al*., 2013). The identification of bacterial isolates exhibiting resistance to contaminated environments and antifungal activity is crucial for understanding their adaptive potential and exploring their applications in phytopathogen biocontrol. *Bacillus* species have garnered significant research interest due to their ability to produce a diverse array of bioactive compounds, including antibiotics, cell wall-degrading enzymes, and secondary metabolites with antifungal properties (Stein, 2005). Key metabolites such as surfactins, iturins, and phenazines exhibit strong antifungal activity by disrupting fungal cell membranes or inhibiting vital biochemical processes (Ongena & Jacques, 2008).

Among the *Bacillus* species, *B. subtilis* and *B. amyloliquefaciens* are particularly well-known for their antifungal properties and have been extensively utilized as biocontrol agents in agroecosystems. Their ability to form spores and withstand extreme environmental conditions, including contaminated soils, makes them promising candidates for application in regions exposed to industrial pollutants (Chandrasekaran & Chun, 2016). Furthermore, these bacteria contribute to plant growth by producing phytohormones and enhancing nutrient availability, thereby reinforcing their role in sustainable agricultural practices (Idriss *et al*., 2002). The relevance of *Bacillus* isolates lies in their potential to serve as biological pesticides, offering an environmentally sustainable and economically viable alternative to chemical pesticides, which are often associated with adverse effects on ecosystems and human health (Ongena & Jacques, 2008). In regions where soil contamination is prevalent, the capacity of *Bacillus* species to tolerate stress conditions while maintaining their biocontrol efficacy is of particular importance.

MATERIALS AND METHODS

Soil samples were obtained from a depth of 5–10 cm using a sterile spatula and placed into sterile containers. To maintain microbial viability, samples were stored at 4°C and processed within 24 hours. For microbial isolation, 1 g of soil was suspended in 90 mL of sterile physiological saline solution (0.9% NaCl). A series of dilutions (10^{-1} to 10^{-5}) was prepared by transferring 1 mL of the suspension into 9 mL of sterile diluent under aseptic conditions (Madigan *et al*., 2019). From the appropriate dilutions, 100 µL aliquots were plated onto Nutrient agar media using the spread plate method. The inoculated plates were incubated at 37°C for 48 hours. Following incubation, colony-forming units (CFUs) were counted (Lee *et al*., 2021). Distinct colonies were selected based on morphological traits, such as size, shape, pigmentation, and texture, and further purified by streaking onto fresh agar plates. For preliminary identification, Gram staining was performed.

A total of 47 bacterial isolates were tested for antifungal activity against 10 phytopathogenic fungi. For each phytopathogenic fungi, 12 Petri dishes were prepared with Nutrient agar (NA) and Potato Dextrose agar (PDA). Wells were made in the agar, and each was filled with 40 µL of bacterial suspension. Plates were incubated at 25°C for 72 hours, and inhibition zones were measured (Balouiri et al., 2016). For secondary screening, isolates were cultured in nutrient broth, centrifuged, and the supernatant and biomass were stored for further testing. The phytopathogenic fungi were inoculated onto NA and PDA plates, and bacterial supernatant was added to the wells. Plates were incubated at 25°C for 5–7 days, and inhibition zones were observed.

RESULTS AND DISCUSSION

The cultural characteristics of 47 bacterial isolates were assessed, revealing variability in colony color, surface texture, and edge morphology. The majority of isolates exhibited a milky-white color, with occasional light-yellow variations; only two isolates displayed pink colonies, characterized by a smooth texture and irregular edges (Figure 1). Gram staining revealed a violet-blue coloration of the isolates under oil immersion, indicating that the isolates were Gram-positive bacteria (Figure 2). The retention of the crystal violet stain, due to the presence of a thick peptidoglycan layer in the cell wall, prevents decolorization by ethanol. Morphologically, the bacteria were identified as rod-shaped.

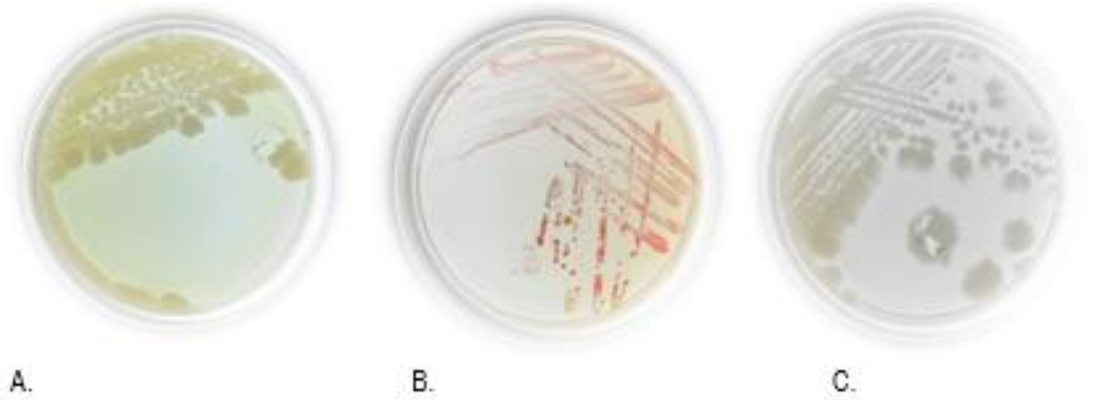

Figure 1. Macroscopic characteristics of: A. Isolate R1-3, B. Isolate R2-2, C. Isolate R4-9

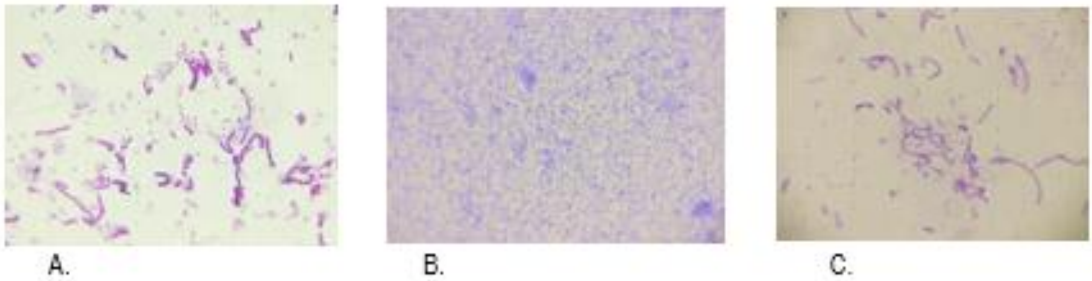

Figure 2. Microscopic characteristcs of: A. Isolate R1-3, B. Isolate R2-2, C. Isolate R4-9

Macroscopically, *Bacillus* colonies can be readily identified by their distinct size, shape, and texture. These colonies generally appear large, round, and dry, with irregular edges, and display characteristic pigmentation ranging from white to cream. While the texture can be smooth, it is more commonly dry and rough, a trait associated with their ability to undergo sporulation (Madigan *et al*., 2019). *Bacillus* species are classified as Gram-positive, aerobic or facultatively anaerobic, catalase-positive, rod-shaped bacteria. They are typically observed as individual cells or in chains, with an average cell length of 2 to 5 µm and a width between 0.5 and 1.2 µm. Due to their capacity for endospore formation, *Bacillus* spp. exhibit resilience to harsh environmental conditions and are prevalent across a broad spectrum of habitats, including soil (Miljaković *et al*., 2020). The spores produced are typically oval or spherical in shape and may be located centrally, subterminally, or terminally, depending on the specific species.

The isolation and quantification of bacteria from contaminated soil represent critical processes in microbiological research, as they provide valuable insights into the microbial community and its role in pollutant degradation. Bacteria, particularly those belonging to the genus *Bacillus*, are of significant importance due to their ability to degrade both organic and inorganic substances, including heavy metals and toxic chemicals. The isolation of these microorganisms facilitates the identification of potentially beneficial species for biological decontamination and allows for the assessment of contamination's impact on the soil's microbial composition. Several factors influence the microbial population in soil, with temperature being a key abiotic factor that affects other sub-factors constituting the soil microbiome, irrespective of climatic conditions and anthropogenic modifications. The ongoing effects of climate change and global warming are altering microbial community dynamics in soils, highlighting the growing importance of microbiological studies in diverse terrestrial ecosystems for understanding the composition and functioning of microbial communities.

The bacterial abundance results (Table 1), expressed in CFU per gram of dry soil, reveal significant seasonal variations. Specifically, the highest abundance was recorded in spring (6.02 Log_{10} $CFUg^{-1}$), followed by autumn (5.83 Log_{10} $CFUg^{-1}$), summer (5.19 Log_{10} $CFUg^{-1}$) and the lowest in winter (5.04 Log_{10} $CFUg^{-1}$). These variations can be attributed to various ecological and climatic factors. Soil properties influencing thermal conductivity, the duration of heating, and the temperatures achieved during soil heating are critical determinants that drive changes in soil microbial communities (Barreiro *et al*., 2020).

Table 1. Abundance of *Bacillus* bacteria in the collected soil samples (Log_{10} $CFUg^{-1}$).

Season	Log_{10} $CFUg^{-1}$
Spring	6.02
Summer	5.19
Autumn	5.83
Winter	5.04

During the summer months, elevated temperatures and humidity promote bacterial growth by activating microbial metabolism and increasing enzyme activity, which leads to a higher bacterial population in the soil. Spring is characterized by a slight decrease in temperature, but it also brings an increase in moisture due to spring rainfall, further supporting bacterial proliferation. In natural systems, fluctuations in temperature and moisture often occur simultaneously, influencing microbial responses through their interactions (Cruz-Paredes *et al*., 2021). In the study conducted by Pietikäinen *et al*. (2005), bacterial growth exhibited a positive trend at temperatures ranging from 25-30°C, attributed to enhanced respiration rates. In contrast to the spring months, bacterial abundance in autumn was significantly lower (5.83 Log_{10} $CFUg^{-1}$). This decline may be attributed to microbial stress induced by decreasing temperatures and humidity, as well as increased evaporation post-summer. The winter season typically presents the lowest bacterial population, as a result of the substantial reduction in microbial metabolism caused by low temperatures and soil freezing. According to the data obtained in this study, bacterial abundance during winter was measured at 5.04 Log_{10} $CFUg^{-1}$. Many bacteria enter a dormant state or form spores to withstand harsh environmental conditions. Snow cover and its persistence significantly impact soil temperature and moisture (Rixen *et al*., 2004), acting as key factors influencing microbial activity during winter (Jefferies *et al*., 2010). Snow cover can insulate the soil from air temperature fluctuations, preventing the physical alterations associated with freezing and thawing (Steinweg *et al*., 2008).

Table 2. Diameter of inhibition zones (mm) of bacterial isolates against test microorganisms.

Isolate	*Plasmopara*	*Erysiphe sp.*	*Monilinia fructigena*	*Penicillium sp.*	*Apergillus ochraceus*	*Alternaria alternata*	*Peronospora*
R1-10	/	/	12.6mm	/	/	/	/
R1-15	/	/	/	/	/	30.65mm	/
R1-19	/	/	/	/	/	/	/
R1-20	/	/	14.17mm	14.33mm	20.52mm	/	/
R2-2	/	15.84mm	/	/	/	14.64mm	/
R4-2	/	15.60mm	/	/	17.25mm	19.44mm	/
R4-3	/	/	/	15.44mm	24.62mm	43.11mm	14.77mm
R4-7	26.82mm	/	17.34mm	18.47mm	23.74mm	/	26.08mm
R4-13	16.46mm	30.35mm	/	20.94mm	18.56mm	/	15.39mm
K+	19.38mm	/	32.83mm	/	/	/	19.18mm

*/ - no inhibition zone

The antifungal activity exhibited by *Bacillus* species plays a crucial role in the biological control of various phytopathogenic fungi. Utilizing *Bacillus* bacteria as a biological agent is of particular significance as it offers a sustainable alternative to chemical fungicides, which are frequently detrimental to the environment and may contribute to the emergence of resistant pathogen strains (Yánez-Mendizábal *et al*., 2012). The results obtained from the study indicate that the isolates demonstrated antifungal activity against the seven test microorganisms (Table 2). The largest inhibition zones were observed for the bacterial isolate R4-13 against *Erysiphe* sp. (30.35 mm), and for isolates R1-15 and R4-3 against *A. alternata*, with inhibition zones measuring 30.65 mm and 43.11 mm, respectively (Figure 3).

Twenty percent of the bacterial isolates demonstrated antifungal activity against the test microorganism *Plasmopara*, while thirty percent exhibited antifungal activity against *Erysiphe*, *Monilinia fructigena*, and *Peronospora*. Forty percent showed activity against *Penicillium* sp. and *A. alternata*, and fifty percent of the *Bacillus* isolates exhibited antifungal activity against *A. ochraceus*. Biological control represents an environmentally sustainable alternative to chemical pesticides and offers an effective method for plant pathogen management. The extensive use of chemical agents has been associated with adverse effects on both the environment and human health. Furthermore, resistant fungal populations are increasingly being identified in intensively managed agro-ecosystems. To mitigate the risks associated with chemical fungicides, antagonistic microorganisms, such as *Bacillus subtilis*, have emerged as a promising alternative. This bacterium has shown considerable potential in inhibiting the growth and sporulation of a broad spectrum of plant pathogenic fungi (Schisler *et al*., 2004). Volatile organic compounds (VOCs) produced by *Bacillus* species demonstrate considerable antifungal activity against various fungal pathogens, including *Monilinia fructigena*. The inhibition rates observed ranged from 47.9% to 84.8%, with the highest inhibition recorded at 84.8% against *M. fructigena* (Guo *et al*., 2020). In a study by Mardanova *et al*. (2016), *Bacillus* species exhibited a 72% inhibition of *Monilinia fructigena* growth. Similarly, *Bacillus* species were found to inhibit the growth of *A. alternata* in research conducted by Ruiz-Sánchez *et al*. (2016), which evaluated the antifungal effects of *Bacillus* bacteria against *A. alternata*. The results obtained in this study are consistent with those reported in the literature. A study conducted in 2024 investigated the antifungal properties of *Bacillus* species isolated from the Brazilian tropical and semi-arid regions as effective antagonists against the fungus *Erysiphe*. During the primary screening, in which bacterial suspensions were applied to detached grapevine leaves, six *Bacillus* strains demonstrated a reduction in disease symptoms exceeding 70.0% (dos Santos *et al*., 2024). Furthermore, a 2020 study evaluated the antifungal activity of *Bacillus* bacteria and β-glucanase against *Aspergillus ochraceus*. The study aimed to uncover their antifungal potential and elucidate the underlying mechanism of action. The findings revealed that bacterial supernatants with a molecular weight (Mw) greater than 10 kDa exhibited superior antifungal activity, which was associated with an increase in chitin content and reactive oxygen species (ROS), alongside the inhibition of mycelial growth and spore germination of *A. ochraceus*.

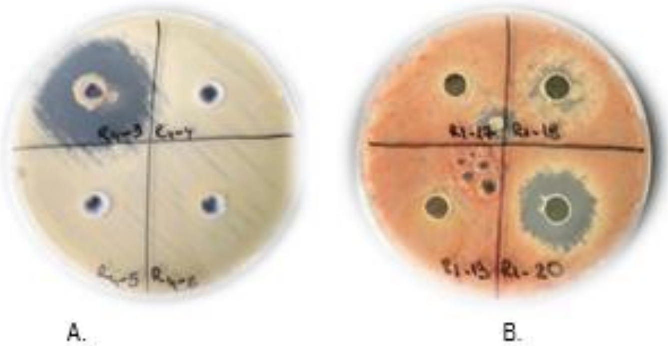

Figure 3. Antifungal activity of: A. Isolate R4-3 against *A. alternata*, B. Isolate R1-20 against *A. ochraceus*

Additionally, these supernatants were shown to activate the CWI signaling pathway at the molecular level, influencing the secondary metabolism of *A. ochraceus*. The antifungal stability and activity of the supernatants were evaluated, with the antifungal efficacy reaching 97.1% (Zhao *et al*., 2022).

The antifungal activity of *Bacillus* isolates is attributed to both their intracellular and extracellular fractions. Notably, the isolates R4-3 and R1-20 exhibited significant antifungal activity from the intracellular fraction against *A. alternata* and *A. ochraceus* (Figure 3). Among the various *Bacillus* species, *Bacillus subtilis* is prevalent and commonly utilized as a biological agent due to its ease of isolation and cultivation (Zhang *et al*., 2016). *B. subtilis* is extensively employed in agricultural practices for the control of fungal diseases, owing to its robust antifungal properties in soil and its adaptability (Chandrasekaran & Chun, 2016). It is well-established that *B. subtilis* produces antifungal enzymes, such as chitinase, cellulase, and β-1,3-glucanase, which degrade fungal structural polymers (Yánez-Mendizábal *et al*., 2012). Additionally, lipopetides synthesized by *Bacillus* species have been shown to exhibit low ecological toxicity and high biodegradability, making them a more environmentally sustainable alternative to chemical pesticides (Roy *et al*., 2018). In agricultural settings, *Bacillus* bacteria are used to manage tomato diseases caused by *Alternaria*, effectively reducing the reliance on chemical fungicides (Köhl *et al*., 2019). A study by Awan *et al*. (2023) aimed to evaluate the antifungal activity of extracellular and intracellular metabolite fractions from *Bacillus subtilis* against *Alternaria*. The results revealed a significant reduction in fungal biomass with extracellular metabolite fractions (69%-98%), while intracellular metabolite fractions achieved a reduction of 85%.

CONCLUSION

In conclusion, this study demonstrates the significant antifungal potential of bacterial isolates from the *Bacillus* genus, particularly those collected from contaminated soil in the Bitola region (REK). The seasonal variation in bacterial abundance indicates that temperature and humidity play crucial roles in bacterial activity, with higher populations observed during spring and lower populations in winter due to reduced temperatures. The antifungal activity of the isolates was found to vary, with notable inhibition zones against a range of phytopathogenic fungi, including *Erysiphe*, *Alternaria alternata*, and *A. ochraceus*. Statistical analysis revealed that 50% of the *Bacillus* isolates exhibited antifungal activity against *A. ochraceus*, underscoring their effectiveness as natural agents for controlling plant diseases.

Furthermore, the study emphasizes the superior antifungal activity of intracellular bacterial fractions, suggesting that metabolites produced by these bacteria are key contributors to pathogen inhibition. These findings highlight the potential of *Bacillus* spp. as sustainable biocontrol agents for managing fungal diseases in agriculture, offering an eco-friendly alternative to chemical pesticides. Such microbial-based strategies could play a pivotal role in the development of integrated pest management systems, contributing to more sustainable agricultural practices.

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MICROBIAL ABUNDANCE IN SOIL AFTER HERBICIDE LUMAX APPLICATION

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ABSTRACT

Lumax 537.5 SE is a selective herbicide that suppresses all types of annual and broadleaf weeds as well as most perennial broadleaf weeds in maize. Although the use of herbicides has a positive impact on weed control in the crop production, it also leads to environmental degradation in the form of soil and groundwater contamination. Microorganisms, as a living component of the soil, play an important role in soil fertility and represent the potential of pesticide tolerance in the environment. The aim of the study was to determine the microbiological activity of the soil after the application of herbicides (Lumax) and to isolate bacteria that are able to grow in the presence of herbicides. The number of bacteria, fungi and actinomycetes determined by the agar plate method increased compared to the control at 10 days after herbicide application. Seven bacterial isolates were isolated from the herbicide-treated soil sample using the enrichment method. The tolerance of the bacterial isolates was determined by growth on nutrient agar medium enriched with different herbicide concentrations. Two isolates (A1 and D1) showed high tolerance to the herbicide and grew best at an initial herbicide concentration of 25 mg/l. These bacterial isolates could have potential applications in the bioremediation of soil and water contaminated with the Lumax herbicide.

Key words: *microorganisms, Lumax, soil, microbial abundance*

INTRODUCTION

Soil is an extremely complex physical, chemical and biological system that serves as a habitat for microorganisms (Dischinger et al. 2009). The microbial populations in the soil and the microbial diversity are important parameters for the quality of ecosystem (Roesch et al., 2007). Therefore, the measurement of soil microbial abundance is necessary to understand the role of microbial population in the soil environment (Zhang et al., 2017). Several studies have addressed the role of microorganisms in the transformation of organic residues, nutrient cycling, plant growth-promoting mechanisms and symbioses in soil (Shay et al., 2015; Tahjib-Ul-Arif et al. 2018; de Souza et al., 2015).

When evaluating microbial processes in agricultural soils, various abiotic and biotic factors are taken into account. One of them is the use of agrochemicals such as fertilizers and pesticides. The application of pesticides, including insecticides, fungicides, herbicides, etc. (Braide et al., 2017), is one of the most important practices in crop production (Meena et al., 2020). However, the widespread use of pesticides leads to environmental degradation (Onder et al., 2011). Due to their stability, persistence and accumulation in soil, surface and groundwater, pesticides are responsible for the pollution of ecosystems (Kellar et al., 2014). Despite the fact that pesticides are designed to act against specific pathogenic microbial and pest populations, several reports have addressed the additional effects on non-target microbial taxa (Staley et al., 2015). In addition, pesticide residues can be found in various products such as fruit juices, beverages, milk and animal feed (Nicolopoulou-Stamati et al., 2016), leading to concerns about the impact on public health (Buscail et al., 2015; Lu et al., 2015).

Thus, it is imperative to find methods for the removal of pesticide residues from the ecosystem and to improve the environmental quality.

Several conventional techniques were proposed for the removal of pesticides in soil. However, these techniques are characterized by low efficiency, high cost of operation process and formation of hazardous intermediates (Javaid et al., 2016). On the other hand, the degradation of xenobiotics using microbial populations is a process known as bioremediation; it is a low-cost and environmentally friendly method for pollutants removal from various environments (Doolotkeldieva et al., 2018). Microorganisms are able to use pesticides as sole energy and carbon sources (Abatenh et al., 2017) and transform them to non-toxic compounds (Jan et al., 2014). The main transformers of pesticide residues in soil are bacteria, fungi and actinomycetes (Diez, 2010). Therefore, isolation of microorganisms from polluted locations and their testing under laboratory conditions is one of the crucial steps in the biological removal of pesticides (Ahmad et al., 2018).

The aim of this paper was the estimation of microbial abundance in soil after application of herbicide Lumax and selection of bacteria able to grow on herbicide as a sole carbon and energy source.

MATERIAL AND METHODS

The study was conducted in an open field at Kakanj (Central Bosnian Canton, Bosnia and Herzegovina) in spring 2019, when the application of herbicide Lumax 537.5 SE (a combination of mesotrione, S-metolachlor and atrazine) in the concentration of 3.5 l/ha was performed in order to destroy weeds in soil under corn. A control soil sample was taken before the herbicide treatment. Additional soil sampling (0-20 cm) was conducted ten days after herbicide application. In order to provide a representative sample, samples were collected from several points throughout the field.

Microbial abundance in soil was determined using the agar plate method. The total number of bacteria was estimated using tryptic soy agar (Torlak, Serbia), fungal abundance on Rose Bengal streptomycin agar (Peper et al., 1995), ammonifying bacteria and microorganisms on nutrient agar (Torlak, Serbia), and actinomycetes on starch-ammonia agar. All experiments were performed in triplicate. Microbial abundance was expressed as colony forming units (CFU) per gram of soil.

Herbicide-tolerant bacteria were obtained using a modification of Talaie et al. (2010) method, with the addition of herbicide Lumax at final concentrations of 0.5; 1.0; 2.0; and 4.0 % (v/v), using nutrient agar. Bacterial isolates were purified, stored at 4°C until further experiments.

Growth of bacterial isolates was examined on nutrient agar supplemented with Lumax at final concentrations of 1.3; 2.2; 3.5; 5.4; and 10.0 g/l (v/v). After incubation at 30°C for 5 days (Binder, Germany), growth rate was measured (0 without growth; + slow growth; ++ moderate growth; +++ intensive growth). The isolates with most intensive growth were chosen for examination of bacterial growth in the presence of Lumax as sole carbon and energy sources. Using pure cultures of bacterial strains (10^8 CFU/ml), inoculation of mineral salt medium (Talaie et al., 2010) supplemented with various Lumax concentration (25; 125; 250; and 500 mg/l) was performed. Bacterial growth was measured spectrophotometrically (T70 Ltd. Instruments, UK) at the start of the experiment, and subsequently after 8; 24; 48; 72; 96; 120; and 144 h.

RESULTS AND DISCUSSION

The obtained results showed a different abundance of microbial groups in samples. Microbial abundance of various groups of microorganisms after Lumax application was presented in Table 1.

Table 1. Abundance of microorganisms in soil

Microbial group	Control	Lumax-treated soil
	x 10^5 CFU/g	
Total number of bacteria	100.0	190.0
Ammonifying bacteria and microorganisms	120.0	170.0
Actinomycetes	1.5	1.6
Fungi	0.3	1.0

The results showed that after ten days of incubation in Lumax treated soil, an increase of microbial abundance was noticed. In the control variant, the total number of bacteria reached 1.0x10^7 CFU/g, while in the Lumax treated soil the total number was almost duplicated, 1.9x10^7 CFU/g. The increase of ammonifying bacteria and microorganisms' population after herbicide treatment was approximately 42% (from 1.2x10^7 CFU/g in control to 1.7x10^7 CFU/g after treatment. Differences in actinomycetes population number were not observed in our research. A high increase of fungal population after herbicide application was observed (from 0.3x10^5 CFU/g in the control, to 1.0x10^5 CFU/g after herbicide treatment).

Seven different bacterial isolates were isolated from soil treated with Lumax. Morphological characterization of bacteria was presented in Table 2.

Table 2. Morphological characterization of bacterial isolates

Isolates	Herbicide concentration (%)	Colony shape	Colony colour	Colony diameter (mm)	Cell shape	Sporulation	Gram staining
B1	1.0	round	white	1.0	rod	+	+
D2	4.0	round	white	3.0	rod	+	+
A1	0.5	round	white	2.5	rod	+	+
C1	2.0	round	yellow	0.8	rod	-	+
A1/1	0.5	round	white	2.8	rod	-	-
B2	1.0	round	Yellow	1.0	rod	+	+
D1	4.0	round	Yellow	1.0	rod	+	+

The results presented in Table 2 showed that most of isolates were characterized as rod spore-forming Gram-positive cells.

To determine the growth rate, the pure cultures of bacterial isolates were used to inoculate nutritional agar supplemented with varying amounts of herbicide. The results of this experiment were presented in Table 3.

At the lowest initial concentration of herbicide (1.3 g/l), intensive growth was presented in four bacterial isolates. Further increase of herbicide concentration in agar was followed by a decrease of bacterial growth rate. At a concentration of 5.4 g/l, growth was noticed only in A1 and D1. These isolates were selected for further research. Their growth rates were further examined in conditions of increased herbicide concentration, up to 500mg/l. The growth of isolates A1 and D1 were monitored for six days.

Table 3. Growth of bacteria on nutrient agar supplemented with herbicide Lumax

Isolate	Herbicide concentration (g/l)	Growth rate	Isolate	Herbicide concentration (g/l)	Growth rate	Isolate	Herbicide concentration (g/l)	Growth rate
B1	1.3	+++	D2	1.3	++	A1	1.3	+++
	2.2	++		2.2	++		2.2	+++
	3.5	+		3.5	++		3.5	+
	5.4	0		5.4	0		5.4	+
	10.0	0		10.0	0		10.0	0
C1	1.3	++	A1/1	1.3	++	B2	1.3	+++
	2.2	+		2.2	+		2.2	++
	3.5	+		3.5	+		3.5	+
	5.4	0		5.4	0		5.4	0
	10.0	0		10.0	0		10.0	0
D1	1.3	+++						
	2.2	+++						
	3.5	++						
	5.4	+						
	10.0	0						

Legend: 0 without growth; + slow growth; ++ moderate growth; +++ intensive growth

Bacterial growth (OD_{600}) of the selected isolates A1 and D1 depended on initial herbicide concentrations and time of sampling and was presented in Tables 4 and 5.

Table 4. Growth of isolate A1 in mineral liquid medium with Lumax as sole C and energy sources

Herbicide concentration (mg/l)	Time of sampling (h)							
	0	8	24	48	72	96	120	144
25	0.120	0.098	0.087	0.132	0.158	0.174	0.135	0.111
125	0.124	0.114	0.093	0.090	0.098	0.125	0.149	0.127
250	0.130	0.121	0.128	0.111	0.102	0.106	0.094	0.090
500	0.125	0.118	0.111	0.097	0.077	0.064	0.062	0.060

At the lowest initial concentration of Lumax (25 mg/l), a decrease of optical density (OD) of isolate A1 up to 24 h of incubation was registred. Further incubation resulted in an increase of OD, with maximal value after 96 h of incubation (0.174). The end of incubation was characterized by a decrease of OD. Initial decrease of OD in the variant with 125 mg/l was followed by an increase, with maximal OD after 120 h (0.149). At initial concentrations of 250 and 500 mg/l, a constant decrease of A1 growth rate was noted.

For isolate D1, the initial rapid decrease of OD at a concentration of 25 mg/l up to 24 h was recorded; further incubation resulted in an increase of OD, with maximum value after 96 h (0.203). At a concentration of 125 mg/l, initial decrease of OD up to 72 h was followed by increase up to the end of incubation. In most of analyzed samples, the supplementation of mineral liquid medium with Lumax at concentrations of 250 and 500 mg/l caused decrease of D1 growth rate, especially in treatment with the highest Lumax concentration.

Table 5. Growth of isolate D1 in mineral liquid medium with Lumax as sole C and energy sources

Herbicide concentration (mg/l)	Time of incubation (h)							
	0	8	24	48	72	96	120	144
25	0.153	0.121	0.099	0.168	0.188	0.203	0.198	0.155
125	0.135	0.108	0.085	0.081	0.077	0.079	0.095	0.105
250	0.144	0.134	0.121	0.129	0.131	0.115	0.110	0.114
500	0.140	0.134	0.120	0.101	0.087	0.071	0.067	0.067

The presented measurements reported that herbicide application had a stimulatory effect on microbial abundance in soil. Those results are in accordance to Baćmaga et al. (2014) who found that the application of Lumax has had a great impact on fungal prevalence in soil. The same authors suggested that an increase in β-glucosidase activity may be associated with herbicide treatment. In contrast, Borowik et al. (2017) claimed that active compounds of Lumax have had a negative impact on the colony development index of bacteria, actinomycetes and fungi. Baćmaga et al. (2015) found the increase in actinomycetes population after Lumax 537.5 SE application, which is confirmed in our research. Furthermore, a broad spectrum of bacterial strains is capable of conversion and degradation of herbicides (Akbar and Sultan, 2016; Jabeen et al., 2015). Moreover, degradation pathways of herbicides are thoroughly described (Ye et al., 2018; Jaiswal et al., 2017). Our results suggested that soil microorganisms are able to use active compounds of Lumax as a sole carbon and energy sources. Martins et al. (2007) described the capability of bacteria to use S-metolachlore as unique carbon and energy source, and the most pronounced ability was expressed by *Enterobacter aerogenes* and *Pseudomonas alcaligenes*. Using *Paracoccus* and *Aquamicrobium* fuel cells, degradation of metolachlor was observed (Li et al., 2019). Olchanheski et al. (2014) showed that *Escherichia coli* strain DH5-α has an ability to use mesotrione as an energy and carbon source.

CONCLUSION

According to the presented data, we may conclude that the application of Lumax led to the increase of microbial abundance in soil under corn. The most pronounced effects were recorded in the total number of bacteria and fungi. Among bacterial isolates obtained from soil supplemented with herbicide Lumax, isolates A1 and D1 were able to grow on nutrient agar with the addition of 5.4 g/l Lumax. Our results showed an increase in optical density (bacterial growth) during incubation in mineral salt medium, which indicates the potential application of these isolates in bioremediation of soils polluted by Lumax`s active compounds. These are preliminary results, opening the door for further investigation of the tested isolates.

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SECTION 4

CLIMATE CHANGES AND NATURAL RESOURCE MANAGEMENT

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ALTITUDE AS A FACTOR ON THE AMPELOMETRIC CHARACTERISTICS OF THE LEAVES OF VRANEC VARIETY

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ABSTRACT

One measure to mitigate the climate change impact in grape production is the selection of a higher altitude location for vineyards. In that direction, the aim of this study was to investigate how does the altitude affects the ampelometric characteristics of the leaves of the wine grape variety Vranec. Findings demonstrate that the terroir of the specific microlocation related to the altitude of the vineyard has a mathematically proven influence on the leaf ampelometric descriptors. This is especially expressed in the vineyard located in Skopje, as a location with highest vineyard altitude among the studied sites. The research included three different vineyards located around Skopje, Veles and Gevgelija. According to the obtained results, the vineyards in Veles and Gevgelija are characterized by relatively similar values and a noticeable tendency of growth in the vineyard located on higher altitude. Comparative statistical analysis reveals that the absolute values of the indicators length of vein N1, N2, N3 and N4 in the vineyard located around Skopje, have higher values compared to the same parameters of the vineyards in Veles and Gevgelija. In addition, the length petiole sinus to upper lateral leaf sinus and length petiole sinus to lower lateral leaf sinus from both halves of the leaf in the vineyard located around Skopje, have higher values compared to the same parameters of the vineyards in Veles and Gevgelija. However, no significant differences between the size of the angles α and β (OIV code 607 and 608) for all vineyard locations were determined in this study.

Key words: *Vineyard location, ampelometric characteristics, altitude, Vranec, ampelometric descriptors.*

INTRODUCTION

In the ampelographic characteristic of each variety, the botanical description of the leaf takes a central place. It is known that by the morphological characteristics of the leaf, each grape variety which has ever been described can be identified with great accuracy. In distinguishing the vine as a species, the main organ described by Carl Linnaeus in 1753 was the leaf petiole (Kovachev 1982). The size and area of the vine leaves have been determined by a number of authors who consider that its significance as an ampelographic organ is indisputable (Stoev, Raduchev 1942-1943, Galet, 1967, 1968; Klimenko et al., 1997; Slavcheva 1990; Zhenhua, Guoguang, 1991). According to Ortiz et al., (1990) the measurement of selected leaf parameters is an effective method for recognizing Vitis biotypes. In the ampelographic methods, computer systems are increasingly being used to process the obtained experimental data (Schneider, Zeppa, 1988). The problem of recognizing Vitis biotypes in the existing large varietal diversity and polymorphism in the vine is substantiated by Sotes et al., (1986). According to Panarina (1967) the size of the leaves of the same vine varieties varies significantly depending on the place of cultivation and meteorological conditions. Despite this, certain differences between them according to given traits are maintained. Troshin et al., (1998) assessed the taxonomic relationships between the varieties from V.v.s.p. balcanica Negr. and V.v.s.p. meridionali – balcanica Trosh. based on morphometric characteristics of the leaves and found that there is no differentiation among Macedonian, Bulgarian and Greek varieties in this group. The aim of this study is to establish the influence of altitude on the botanical characteristics of the leaf in the Vranec variety.

MATERIALS AND METHODS

The botanical description of the leaf was carried out over a five-year period with Vranec vines grown in three regions in R. Macedonia at different altitudes: Gevgelija – 50 m, Veles – 280 m, and Skopje – 595 m. The vines were trained by a bilateral guyot system. To avoid inaccuracies in the description of the leaf of the variety Vranec, it is accepted as typical leaves to consider those naturally positioned from the 9th to the 12th node, counted from the base of the cane to the top of the vine shoots (Bulgarian Ampelography 1990; Bozhinović 2010; Roychev 2012). All leaf descriptors were measured, described, and analyzed comparatively according to the published OIV methodologies (OIV April 2001), CODES shown on (Fig. 1). The obtained experimental data were mathematically processed using two-factor dispersion analysis modules and Duncan's criterion with the statistical softer SPSS 17.

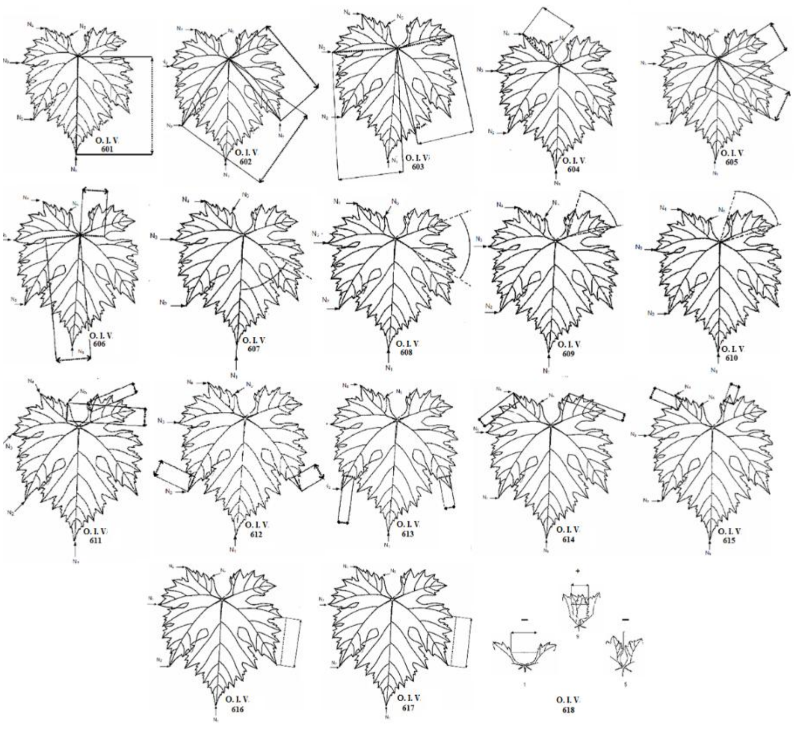

Figure 1. OIV Code for leaves ampelographic description.

RESULTS AND DISCUSSION

The multipoint comparative analysis of the ampelometric indicators of the leaf in the better-developed part of the Vranec variety shows that the length of vein N1 (CODE 601) is proven to be the longest in the Skopje area – 121.4 mm, compared to Veles – 105.5 mm and Gevgelija – 100.9 mm (Table 1). Two groups of significance were formed – a and b. The same applies to the length indicators of petiole sinus to upper and lower lateral leaf sinus (CODES 605 and 606) and the length of vein N4 (CODE 604). The differences in their values are significant. The trend is also maintained in the length petiole sinus to upper and lower lateral leaf sinus

(CODES 605 and 606), with their absolute values being closer. No significant differences were found between the sizes of angles α and β between N1 and N2 (CODES 607 and 608), as only one group of statistical significance was found.

Table 1. Multipoint Comparative Analysis of Ampelometric Descriptors of the Better-Developed Part of the Leaf in the Vranec Variety

Variants	O.I.V. CODE Nº and Descriptor							
	601	**602**	**603**	**604**	**605**	**606**	**607**	**608**
	Mature leaf: length of vein N1	Mature leaf: length of vein N2	Mature leaf: length of vein N3	Mature leaf: length of vein N4	Mature leaf: length petiole sinus to upper lateral leaf sinus	Mature leaf: length petiole sinus to lower lateral leaf sinus	Mature leaf: angle between N1 and N2 *measured at the first ramification. *Code Nos OIV 601 and OIV 602	Mature leaf: angle between N2 and N3 measured at the first ramification
Gevgelija	100.9 b	89.3 b	63.6 b	39.4 b	46.2 b	43.7 b	55.7 a	59.8 a
Veles	105.5 b	92.2 b	64.3 b	39.2 b	53.4 a	44.3 b	54.1 a	61.0 a
Skopje	121.4 a	112.0 a	80.1 a	50.4 a	55.2 a	54.2 a	55.6 a	63.0 a

Some variety in variants is observed in the variation of the next two indicators: the γ angle between N3 and N4 (CODE 609) and the angle between N3 and the tangent between petiole point and the tooth tip of N5 (CODE 610), shown in Table 2. In the first case, leaves from vines in Skopje and Gevgelija are in one group of significance (a), and in the second – Veles and Skopje. For the length of vein N5 (CODE 611), the length of tooth of N2, the length of tooth of N4, and the width of tooth N4, the leaves from the vineyard around Skopje have proven to have the highest values. Only for the width of tooth N2 there are no significant differences between the three locations (variants). The leaves in the vineyard around Gevgelija have relatively more tooth between the tooth tip of N2 and the tooth tip of the firs secondary vein on N2 including the limits (CODE 616), but the length between the tooth tip N2 and the tooth tip of the first secondary vein of No2 (CODE617) is longer in the leaves samples from Skopje (Table 3). The Opening/overlapping of petiole sinus of the leaves are predominantly closed in the leaves from Gevgelija, while in Skopje and Veles, they are mostly open.

Table 2. Multipoint Comparative Analysis of Ampelometric Indicators of the Better-Developed Part of the Leaf in the Vranec Variety

Variants	O.I.V. CODE Nº and Descriptor						
	609	**610**	**611**	**612**	**613**	**614**	**615**
	Mature leaf: angle between N3 and N4 measured at the first ramification	Mature leaf: angle between N3 and the tangent between petiole point and the tooth tip of N5	Mature leaf: length of vein N5	Mature leaf: length of tooth of N2	Mature leaf: width of tooth of N2	Mature leaf: length of tooth of N4	Mature leaf: width of tooth of N4
Gevgelija	60.6 a	79.6 a	19.9 b	11.4 b	13.8 a	8.8 c	12.1 b
Veles	54.3 b	69.3 b	19.0 b	13.2 ab	14.7 a	9.9 b	12.0 b
Skopje	60.6 a	72.5 b	24.7 a	14.2 a	14.4 a	11.9 a	14.3 a

Table 3. Multipoint Comparative Analysis of Ampelometric Indicators of the Better-Developed Part of the Leaf in the Vranec Variety

Variants	O.I.V. CODE Nº and Descriptor		
	616	**617**	**618**
	Mature leaf: number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2	Mature leaf: opening/overlapping of petiole sinus
Gevgelija	4.5 a	51.7 b	-1.8 b
Veles	4.1 b	50.9 b	9.1 a
Skopje	4.3 ab	59.9 a	13.4 a

The obtained results show that the studied ampelometric indicators of the leaves from the vineyard in the Skopje area differ from those in the other two regions. Their absolute values are most often greater and form a separate group of significance. The multipoint comparative analysis of the ampelometric indicators of the less developed part of the leaf in the Vranec variety entirely repeats the outlined trends in their variation depending on the location of the vineyard (Table 4, Table 5). Some differences in the formed groups and the statistical significance of the differences are observed only in the depth of the lower lateral incision, the γ angle between the main veins, the length of notch No. 4, and the number of notches between the lateral main vein and secondary vein No. 2.

Table 4. Multipoint Comparative Analysis of Ampelometric Indicators of the Less-Developed Part of the Leaf in the Vranec Variety

Variants	O.I.V. CODE Nº and Descriptor							
	602	**603**	**604**	**605**	**606**	**607**	**608**	**609**
	Mature leaf: length of vein N2	Mature leaf: length of vein N3	Matur e leaf: length of vein N4	Mature leaf: length petiole sinus to upper lateral leaf sinus	Mature leaf: length petiole sinus to lower lateral leaf sinus	Mature leaf: angle between N1 and N2 *measured at the first ramification *Code Nos OIV 601 and OIV 602	Mature leaf: angle between N2 and N3 measured at the first ramificatio n	Mature leaf: angle between N3 and N4 measured at the first ramificatio n
Gevgelija	84.1 b	63.3 b	38.9 b	46.1 b	11.0 a	55.2 a	63.3 a	58.3 a
Veles	91.5 b	64.9 b	39.1 b	50.5 ab	10.0 a	52.6 a	60.7 a	53.8 a
Skopje	115.5 a	80.0 a	49.7 a	56.0 a	10.0 a	55.7 a	63.4 a	60.5 a

Table 5. Multipoint Comparative Analysis of Ampelometric Indicators of the Less-Developed Part of the Leaf in the Vranec Variety

Variants	O.I.V. CODE Nº and Descriptor							
	610	**611**	**612**	**613**	**614**	**615**	**616**	**617**
	Mature leaf: angle between N3 and the tangent between petiole point and the tooth tip of N5	Mature leaf: length of vein N5	Mature leaf: length of tooth of N2	Mature leaf: width of tooth of N2	Mature leaf: length of tooth of N4	Mature leaf: width of tooth of N4	Mature leaf: number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2
Gevgelija	79.8 a	19.1 b	10.9 b	13.7 a	8.8 b	12.0 b	4.7 a	51.0 b
Veles	69.7 b	18.9 b	12.4 ab	14.6 a	9.7 b	12.1 b	4.2 a	50.7 b
Skopje	72.4 b	24.2 a	13.8 a	14.2 a	11.7 a	13.9 a	4.2 a	

CONCLUSION

Soil and climatic factors related to the altitude of the terrain have a mathematically proven influence on the ampelometric characteristics of the leaf of Vranec variety, manifested by an increase in their size in the Skopje area. In the vineyards around Gevgelija and Veles, they are characterized by relatively close values and a noticeable trend of increase in higher-altitude terrain.

According to the multipoint comparative analysis, the absolute values of the indicators such as the length of vein N1 (CODE601), the length of vein N3 (Code 603), the length of vein N4 (CODE 604), the length of petiole sinus to upper and lower lateral leaf sinus (CODE 605,606) on both halves of the leaves in the vineyard around Skopje, have higher values for the same parameters measured for the leaves in the vineyards in Veles and Gevgelija. There are no significant differences in the size of the angles between N1 and N2 (CODE 607) and angle between N2 and N3 (CODE 608).

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CONTENT OF HEAVY METALS OF SOILS FORMED ON ANDESITE ROCKS FROM THE AREA OF KRATOVO

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ABSTRACT

The investigation aimed to determine the total concentrations of heavy metals (Pb, Cd, Cu, and Zn) and the available forms of copper and zinc in soils developed on andesite rocks in the Kratovo area. Soil samples were digested using a microwave furnace (Ethos Easy model). The available forms of heavy metals were extracted using the DTPA method. Heavy metal concentrations were measured with an Agilent 55 atomic absorption spectrophotometer. The results showed that total concentrations of zinc, lead, and copper in all soil samples were below the reference values, while cadmium levels exceeded the reference values but remained well below the intervention threshold. Available copper ranged from low to high, whereas available zinc was classified between very low and low.

Key words: *andesite rocks, heavy metals, mollic ranker, cinnamonic forest soil*

INTRODUCTION

Andesite is a transitional volcanic rock with a SiO_2 content ranging from 55 to 65% (Гапковски et al. 2007). In our country, andesite occurs in several localities with the largest areas in the Kratovo-Zletovo eruptive area. These andesitic rocks serve as a source material for the formation of some soil's types. From the previous research, it can be concluded that mollic ranker Manuševa (1959) and smolnitza Петковски (1988), are formed on this type of rocks. Ćirić (1953) describes mollic ranker on andesites in Eastern Serbia, while Manuševa (1971) describes mollic ranker, cinnamonic forest soil, brown forest soil and ilimerized soil, on this substrate in Bosnia and Herzegovina. With our research in the Kratovo-Zletovo eruptive area we have determined the formation of mollic ranker and cinnamonic forest soil on andesites. From the literature review, we did not find data on the content of total and available forms of heavy metals in soils formed on andesites.

In this paper are presented data of the mechanical composition, some chemical properties and content of total forms of Pb, Cd, Cu and Zn and available forms of Cu and Zn in soils formed on andesite rocks from the area of Kratovo Republic of Macedonia. On different locations of the area of Kratovo 4 basic pedological profiles were excavated of mollic ranker with the subtypes haplic (prof. N° 1, 5, 6, and 7) and one profile of cinnamonic forest soil with subtype leptic vertic (prof. N°2). The investigated soils are classified according proposed classification of the Republic of Macedonia (Filipovski 2006).

The date of some soil-forming factors of soils formed on andesite rocks from the area of Kratovo are presented on Table 1.

The main goal of this paper is to examine chemical properties, mechanical composition and content of total (Pb, Cd, Cu and Zn) and available forms of Cu and Zn in soils formed on andesite rocks from the area of Kratovo.

Table 1. Some soil-forming factors of soils formed on andesite rocks

Profile Nº	Parent material	Altitude, m	Exposure	Inclination, %	Stonines, %	Occurrence of Outcrops, %	Agri-culture crop
1	Andesite	662	East	10-15	0	0	Alfalfa
2	Andesite	656	East	10-15	0	0	Alfalfa
5	Andesite	588	West	10-15	0	0	Alfalfa
6	Andesite	589	West	10-15	0	0	Alfalfa
7	Andesite	555	West	10-15	0	0	Alfalfa

MATERIALS AND METHODS

Field examinations have been performed according to accepted methods in Former Yugoslavia (Filipovski ed., 1967).

The laboratory analyses have been done according to the standard adopted methods in Former Yugoslavia and Republic of Macedonia, as follows:

Mechanical composition of soil was determined by the pipette method (Resulović ed., 1971); the dispersion of the particles has been done with 0,4N Na-pyrophosphate. The separation of the mechanical elements in fractions has been done by the international classification.

pH (reaction) of the soil solution has been determined with glass electrode in water suspension and in NKCI suspension (Bogdanović ed., 1966).

Easily available forms of P_2O_5 and K_2O were determinate by Al method (Džamić et al., 1996).

The content of humus has been determinate at the base of organic carbon by the method of Tjurin modified by Simakov (Orlov & Grišina, 1981).

Soil samples were digested using a microwave furnace (Ethos Easy model). The available forms of heavy metals are extracted with the DTPA method (Page ed., 1982). Heavy metal concentrations were measured with an Agilent 55 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Data on the mechanical composition and some chemical properties of the tested soils are presented in Tables 2, 3 and 4.

The mechanical composition of soils significantly influences the toxicity and mobility of heavy metals. In lighter soils with lower clay content, the toxicity of heavy metals increases, and they migrate more easily to deeper soil layers, thereby increasing the risk of groundwater pollution.

As shown in Tables 2 and 3, the tested soils are characterized by a heavier mechanical composition, which reduces the risk of phytotoxicity.

Table 2. Mechanical composition of soils formed on andesite rock

Profile Nº	Horizon and depth in cm	In % of fine earth					
		Coarse sand 0.2 - 2mm	Fine sand 0.02 - 0.2mm	Coarse + fine sand 0.02-2mm	Silt 0.002 - 0.02mm	Clay <0.002mm	Silt + clay <0.02mm
1	Ap 0-30	15,1	33,7	48,8	16	35,2	51,2
1	AC 30-60	22	31,9	53,9	16,5	29,6	46,1
1	C/R 60-80						

1	R						
2	Ap 0-30	9,9	25,5	35,4	16,9	47,7	64,6
2	(B)v 30-58	7,4	19,6	27	16,9	56,1	73
2	(B)vC 58-82	16,2	38,6	54,8	20,5	24,7	45,2
2	C/R 82-100						
2	R						
5	Ap 0-35	21,3	41,5	62,8	12,7	24,5	37,2
5	AC 35-69	20	45,6	65,6	11,3	23,1	34,4
5	C/R 69-75						
5	R						
6	Ap 0-29	12,7	51,4	64,1	10,5	25,4	35,9
6	AC 29-54	10,9	53,2	64,1	15	20,9	35,9
6	C/R 54-65						
6	R						
7	Ap 0-23	17,8	44,9	62,7	19,4	17,9	37,3
7	AC 23-41	23,6	43,8	67,4	17,4	15,2	32,6
7	C/R 41-64						
7	R						

Table 3. Textural classes acording Scheffer & Schachtschabel classification

Profile N°	Horizon and depth in cm	Textural classes
1	Ap 0-30	Loamy clay
1	AC 30-60	Loamy clay
1	C/R 60-80	
1	R	
2	Ap 0-30	Heavy clay
2	(B)v 30-58	Heavy clay
2	(B)vC 58-82	Clay loam
2	C/R 82-100	
2	R	
5	Ap 0-35	Sandy clay loam
5	AC 35-69	Sandy clay loam
5	C/R 69-75	
5	R	
6	Ap 0-29	Sandy clay
6	AC 29-54	Sandy clay loam
6	C/R 54-65	
6	R	
7	Ap 0-23	Clay loam
7	AC 23-41	Sandy clay loam
7	C/R 41-64	
7	R	

The tested soils are free of carbonates, which increases the availability of heavy metals. The availability of heavy metals depends on soil reaction (pH). The uptake of numerous heavy metals intensifies with increasing of soil acidity. Out of data presented in Tab. 4, it can be concluded that soil reaction is slightly acid and neutral, due to what it can be expected and reduced availability of heavy metals.

Table 4. Some chemical properties of soils formed on andesite rock

Profile	Horizon and	$CaCO_3$,	Humus,	pH		Easy available, mg/100g soil	
Nº	depth in cm	%	%	H_2O	nKCl	P_2O_5	K_2O
1	Ap 0-30	0,00	5,03	7,15	5,75	4,23	23,78
1	AC 30-60	0,00	2,37	7,08	5,72	2,48	18,68
1	C/R 60-80						
1	R						
2	Ap 0-30	0,00	4,01	6,36	5	1,5	26,33
2	(B)v 30-58	0,00	3,14	6,2	4,96	0,67	22,93
2	(B)vC 58-82	0,00	1,5	6,3	4,94	0,51	15,71
2	C/R 82-100						
2	R						
5	Ap 0-35	0,00	4,04	6,77	5,26	5,78	32,27
5	AC 35-69	0,00	2,17	7,09	5,53	4,97	19,53
5	C/R 69-75						
5	R						
6	Ap 0-29	0,00	4,66	6,75	5,56	3,43	37,79
6	AC 29-54	0,00	2,67	7,07	5,67	2,74	24,2
6	C/R 54-65						
6	R						
7	Ap 0-23	0,00	5,07	6,34	4,41	5,89	21,65
7	AC 23-41	0,00	3,67	6,75	4,92	4,12	19,11
7	C/R 41-64						
7	R						

Content of total forms heavy metals

In Table 5, are shown the data related to the content of total forms of Zn, Cd, Pb and Cu in examined soils. For comparison of the results the Dutch reference standards will be used (Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer 2010). According to Dutch standards: a) if the content of heavy metals is below reference values, agricultural crops can be grown without limitations; b) if the content of heavy metals is between the reference and intervention values, a proper selection of agricultural crops is required, along with continuous monitoring of the available content of heavy metals in the soil and its concentration in plant tissues of agricultural products, and c) if the content of heavy metals is above intervention values, the soil are unsuitable for agricultural production and remediation is necessary.

Table 5. Content of total forms heavy metals in soils formed on andesite rocks

Profile Nº	Horizon and depth in cm	Total content, $mg.kg^{-1}$			
		Zn	Pb	Cu	Cd
1	Ap 0-30	77,23	27,57	11,57	1,10
1	AC 30-60	46,36	22,71	10,11	1,41
1	C/R 60-80				
1	R				
2	Ap 0-30	59,58	43,42	10,01	1,02
2	(B)v30-58	65,09	51,92	12,40	1,71
2	(B)vC 58-82	64,68	29,10	9,28	0,90
2	C/R 82-100				

2	R				
5	Ap 0-35	62,94	36,85	3,89	1,11
5	AC 35-69	56,32	24,26	4,82	1,05
5	C/R 69-75				
5	R				
6	Ap 0-29	60,00	28,16	6,53	1,07
6	AC 29-54	56,95	29,74	4,42	0,84
6	C/R 54-65				
6	R				
7	Ap 0-23	79,21	27,09	6,05	1,08
7	AC 23-41	62,00	24,27	4,23	1,22
7	C/R 41-64				
7	R				
Referent value		***140***	***85***	***36***	***0,8***
Intervene value		***720***	***530***	***190***	***12***

The content of total zinc, lead and copper in all tested soil samples is lower than the reference values, which means that there is no danger of contamination of soil and plants with this heavy metals.

The global average concentration of lead in soils is 35 mg·kg^{-1} soil, while in the surface layers of the soils in Europe, it is 33 mg·kg^{-1} soil (Bowen 1979, cited by Stafilov et al., 2016). The average content of total lead in the surface layer of soil in our country is 34 mg·kg^{-1} soil (Stafilov et al., 2016). From the data in the table, it can be concluded that our data for total lead are within the range of the above cited data.

The content of total zinc in the lithosphere is approximately 80 mg·kg^{-1} soil, while in soils it usually ranges from 10 to 300 mg·kg^{-1} soil, with an average of 50 mg·kg^{-1} soil (Lindsay, 1982, cited by Kastori ed., 1997). Our results (Table 5) are consistent with the global average. The content of total zinc in cinnamonic forest soil from Negotino area was determined by Јекиќ et al. (1972), in range from 24.8 to 48.8 mg·kg^{-1} soil. The results from our research are lower than the average for the surface layer of soils in the country, according to Stafilov et al. (2016) who noted 82 mg·kg^{-1} soil.

According to previous studies, the global average content of total copper in soils is 20 or 30 mg·kg^{-1} soil (Kastori ed., 1997). Based on our data, it can be concluded that the content of total copper in the examined soils which are formed on andesites, is below the global average. In 1972 by Јекиќ et al., determined the total copper content in cinnamonic forest soil from Negotino area, ranging from 26.4 to 30.4 mg·kg-1 soil. The average content of total copper in the surface layer of soils in our country is 27 mg·kg-1 soil (Stafilov et al., 2016).

From Table 5, it can be noted that the total cadmium content in the tested soil is slightly higher than the reference values, but much lower than the intervene values. This note indicates that there is no risk of soil contamination and plant with this metal.

The total cadmium content in uncontaminated areas in the USA ranges from 0.005 to 2.4 mg·kg^{-1} soil, with an average of 0.27 mg·kg^{-1} soil (Holmgren, cited by Kastori ed., 1997). In England and Wales, the average cadmium content was measured at 1.2 mg·kg^{-1} soil, from 2276 soil samples (MCGrath, cited by Kastori ed., 1997). Based on this, it can be concluded that our values for total cadmium are within the range of those of the USA and England.

As mentioned above, the studied soils are formed on andesites. On a similar substrate, i.e. andesite breccia, in the Kratovo-Zletovo eruptive area rigosols formed by the deep plowing of smolnitza. For these soils, Andreevski et al. (2011) report total content of: Zn ranged from 36.36 to 81.40 mg·kg^{-1} soil, Pb from 22.35 to 37.42 mg·kg^{-1} soil, Cd from 0.47 to 1.13 mg·kg^{-1} soil and Cu from 10.34 to 32.39 mg·kg^{-1} soil.

Mitrikeski et al. (2000) determined the content of total forms of heavy metals in cinnamonic forest soil from the Prilep and Kumanovo regions: Cu ranged from 11.16 to 36.03 mg·kg^{-1} soil, Pb from 6 to 23.99 mg·kg^{-1} soil and Zn from 12.21 to 52.04 mg·kg^{-1} soil.

For two cinnamonic forest soil profiles from the Skopje region (arable soil layer), Petkovski et al. (2006) reported total Cu content of 7.4 and 9.4 mg·kg^{-1} soil, total Zn 40.17 and 41.99 mg·kg^{-1} soil and total Pb 9 and 10 mg·kg^{-1} soil. Additionally, for a one surface soil sample from cinnamonic forest soil in the Skopje region (locality Kamnik) Petkovski et al. (2001) noted total content of Cu was 12.3 mg·kg^{-1} soil, total Zn was 75.6 mg·kg^{-1} soil and total Pb was13 mg·kg^{-1} soil.

Content of the available forms of Cu and Zn

Date of available forms of copper and zinc are presented in Table 6. These heavy metals are essential trace elements in plant nutrition and their deficiency can cause growth failure while the greater scarcity can cause extinction of plants. On the other hand, if these trace elements are present in high concentrations can lead to phytotocsicity.

Table 6. Content of available forms heavy metals in soils formed on andesite rocks

Profile N°	Horizon and depth, cm	Available form, mg.kg^{-1}	
		Cu	Zn
1	Ap 0-30	1,21	0,582
1	AC 30-60	1,10	0,416
1	C/R 60-80		
1	R		
2	Ap 0-30	1,60	0,556
2	(B)v 30-58	1,68	0,432
2	(B)vC 58-82	1,72	0,698
2	C/R 82-100		
2	R		
5	Ap 0-35	0,81	0,66
5	AC 35-69	0,73	0,342
5	C/R 69-75		
5	R		
6	Ap 0-29	0,89	0,674
6	AC 29-54	0,76	0,498
6	C/R 54-65		
6	R		
7	Ap 0-23	0,77	0,696
7	AC 23-41	0,86	0,686
7	C/R 41-64		
7	R		
very low		***<0,3***	***<0.5***
low		***0.3-0.8***	***0.5-1.0***
medium		***0.9-0.1,2***	***1.1-3.0***
high		***1.3-2,5***	***3.1-6.0***
very high		***>2,5***	***>6.0***

Out of data presented in the table it can be concluded that 4 soil samples contain low quantities of available copper, 4 soil samples medium and 3 soil sample have high quantities of available copper.

The content of available zinc in 4 soil samples has very low quantities and 7 soil samples have a low content.

On the base of our research, it can be concluded that the quantities of available copper in some soil samples and zinc in all soil samples are not satisfactory.

CONCLUSIONS

Based on the conducted research the following conclusions can be drawn:

Out of the data for total Cu, Cd, Zn and Pb it can be concluded that there is no threat of toxic amounts of heavy metals to the soils and plant.

Quantities of available copper in some soil samples and zinc in all soil samples are not satisfactory and can cause growth failure

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TILLAGE SYSTEMS AND THEIR IMPACT ON CROP YIELD AND SOME PHYSICAL AND CHEMICAL SOIL PROPERTIES

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ABSTRACT

Land cultivation systems through several methods of cultivation in one time continuity, can fulfil very different requirements from agronomic to economic, whereby they are required to provide optimal conditions for sowing, growth and development of plants with the least expenditure of resources. Today, these requirements have been extended in the ecological sense, considering that land is a natural resource that needs to be preserved for future generations. The height of the yield of cultivated plants largely depends on the selected tillage system, and the tillage system represents the connection of individual methods of basic and supplementary tillage into one agrotechnically harmonious whole. The choice of soil cultivation system depends on numerous factors: climate characteristics, soil types and properties, relief characteristics, fertilization system, crop requirements, place in the crop rotation, hereditary basis of the plant species. Based on this, it can be seen that soil and climate are constant factors, and everything else is variable. Therefore, there is no universal land cultivation system for the entire agrosphere, it is regional and sometimes local.

Key words: *tillage system, soil, yield,*

INTRODUCTION

Tillage is a set of technological operations that are the first in a series of agricultural production. Since it accounts for over 30%, and in very difficult conditions even up to 50% of the total energy consumption in crop production, tillage is a demanding operation (Veljić et al., 2008).

Liu et al. (2013) point out that the effects of different tillage systems on soil characteristics and crop yield depend on climatic conditions, soil and crop type. Glinski and Lipiec (1990) found that in wet conditions, soils with a higher content of organic matter could show better resistance to compaction compared to soils with a lower content of organic matter and when applying the same soil treatment systems. In temperate climate regions, as stated by Gruber et al. (2012) and Drury et al. (2013), maize yields obtained under no-till conditions were similar or lower compared to those obtained using conventional tillage in cold and wet climates or on poorly drained soils, where the absence of tillage reduces erosion caused by wind and water (Drury et al., 1999; Puustinen et al., 2005), increases the degree of water infiltration as well as its content in the soil while simultaneously reducing labor and fuel consumption, thus making the no-tillage system more commercially attractive compared to most conventional tillage systems (Moussa-Machraoui et al. al., 2010; Soane et al., 2012). Tillage according to Lal et al. (2007), especially in moderate climatic conditions, aims at accelerated soil heating and water evaporation, weed destruction and temporary improvement of soil physical conditions for plant establishment. However, excessive and long-term processing in this way affects the degradation of the soil structure through a decrease in the stability of soil aggregates, size and porosity, an increase in the compaction of the lower soil layers and the formation of the so-called Plow pans and surface crusting, which reduces infiltration and increases the potential for erosion.

The aim of this paper is to indicate the influence of conventional and conservation treatment on some important properties of the soil as the basic means on which plant production is organized.

The influence of the tillage system on crop yield, soil compaction and the content of organic matter in the soil

Examining the influence of soil tillage system on soil compaction and winter wheat yield, where the research included four tillage systems (conventional tillage, reduced tillage, disc harrowing, and no-tillage) Biberdžić et al. (2020) came to the conclusion that the highest yield of wheat grains was achieved using the conventional tillage system. The achieved yield of wheat when using the mentioned tillage system was significantly higher than the yield obtained in other soil tillage systems. The same authors stated that the highest mean soil compaction (2.47 MPa) was measured in no-tillage (NT) and was significantly higher than in reduced tillage and conventional tillage (CT). Nonsignificant differences were found between the reduced tillage (RT - disc harrowing + seedbed preparation) and reduced tillage system RT1 which means just disc harrowing, and the RT1 and CT systems for mean soil compaction. Similar results were obtained by Wozniak (2013) examining the impact of conventional, reduced tillage and herbicide tillage, pointing out that the highest yield of wheat was obtained with the application of conventional tillage land in the years with the higher total rainfall.

According to Veljić et al. (2008) when applying classical tillage, all operations, ie technical systems, follow each other, with a certain time interval, as a result, tillage can last for several months. By unifying operations, i.e. by creating a modular system, numerous operations are performed with modules, and not with individual technical systems, which leads to a reduction in costs. Each operation within the framework of land cultivation aims to fulfill certain agrotechnical requirements. By performing several operations in one pass using a modular system, all individual operations can be classified into a technology, which with one modular technical system will replace all collective soil processing operations, reducing the degree of soil compaction.

Karlen et al. (2019) pointed out that to be healthy, a soil must have optimum biological, physical, and chemical properties and processes. Reducing tillage has a positive effect on physical, chemical and biological soil properties and crop yields (Aziz et al. 2013; Nunes et al. 2020). Nevertheless, Panasiewicz et al. (2020) state that any type of tillage reduction compared to conventional tillage affects reduced productivity in wheat. On the other hand, in a drier climate, the yields of wheat obtained using conventional and reduced tillage are comparable (Pittelkow et al. 2015).

Šeremešić et al. (2016) researched the influence of conventional and reduced tillage on the content of total and easily accessible organic matter in the soil. At the same time, conventional cultivation in wheat was carried out with a plow at a depth of 25 cm, and in sunflower at 27 cm, while reduced cultivation in wheat was carried out with a heavy disc at 15 cm, and in sunflower with a combined harrowing tool Horsch Terano 3 at 25 cm. The results of the research showed that the total content of organic matter in the soil was higher on the plots where reduced tillage is performed compared to the conventional tillage system. Through statistical analysis, it was determined that there is a significant effect of the processing system and crops on the change in the content of total organic matter. Using reduced tillage, the highest value of labile organic matter soluble in warm water (HWC) in wheat was determined at a depth of 0-10 cm, and in sunflower 10-20 cm, which indicates that the place of plowing and the amount of plant residues affect the accumulation of organic matter. The greater influence on the content of HWC was shown by the depth in relation to the tillage system and the crop. Regression analysis found that with the increase in total organic matter, the content of HWC also increases. The same authors suggest that it would be important to conduct research regarding reduced cultivation and positive effects on the quality of the soil itself. As stated by Marinković et al. (2005), wheat is a plant species that best tolerates less tillage and compacted soil, but it also responds best to the prolonged effect of tillage depth, if a deep arable layer has been achieved. According to Gal et al. (2007) and Thomas et al. (2007), tillage practices may also influence the distribution pattern of soil organic carbon (SOC). The mentioned authors point out that a higher concentration of SOC was observed in the surface layers when tillage was left out compared to plots where conventional tillage was applied. but a higher concentration of SOC in the deeper soil layers of reduced tilled plots where crop stubbles are incorporated through tillage. Alijani et al. (2012) and Obour et al. (2017) stated that conservation tillage practices are an operative management practices to increase SOC unlike Govaerts et al. (2009) and Sainju et al. (2008) who determined that no tillage without crop residue resulted in lesser or no change in SOC.

The influence of tillage systems on soil moisture accumulation and content

The tillage system showed a significant impact on the accumulation and content of moisture in the soil. Accordingly, Marinković et al. (2005) pointed out that the difference in the amount of water in the 0-40 cm layer during the summer on cultivated land is greater by 154,750 to 228,000 l ha-1 compared to untreated land at the same amount of precipitation. In addition to the higher accumulation, the consumption of water for the synthesis of 1 kg of yield is lower, i.e., with compacted soil, 8.9 kg of yield was formed per unit of water consumed, while with loose - processed, the yield was 11.6 kg. Similar results were obtained by Biberdžić et al. (2020) as well as Pansak et al. (2008) who stated that soil moisture is best conserved using conventional tillage systems. Alsayim et al. (2021) in their research (2012-2014) applied treatments of the three tillage systems: disc plow followed by land leveller (DPL), disc harrow followed by land leveller (DHL) and zero tillage with the depth of plowing mostly within the range of 20-25 cm and the harrowing within the range of 8-10. The mentioned authors came to the conclusion that soil moisture content increases with depth and then decreases at specific depths for all treatments during both seasons. In all applied treatments, the highest moisture content in the soil was found at a depth of 15-30 cm, which could be explained as a consequence of soil cultivation. Also, they determined that the totals appear to be nearly equal and this shows that the soil texture (more sand) has the greater effect on soil moisture content than the tillage treatments, which is in agreement with the statements of Nayel et al. (2016) and Blanco-Canqui et al. (2017) which pointed out that the water infiltration was greater in the soil under more intensive tillage (after moldboard plowing) as compared to no tillage, disk and chisel plow. Blanco-Canqui et al. (2017) also concluded based on their research that no-till farming even in the long term (35 yr) may have limited or no positive effect on increasing water infiltration and retention compared with moldboard plow and conventional tillage systems such as disk and chisel plough.

Rusinamhodzi et al. (2011) analyzed the long-term effects of conservation agriculture on corn grain yield under rain-fed conditions, pointing out that corn yield gave higher values in the case of ecologically acceptable agricultural methods (conservation agriculture practices) with lower annual precipitation (< 600 mm), while lower values produced by precipitation above 1,000 mm. Omission of tillage in the plot covered with crop residues resulted in improved soil water retention capacity and bulk density, but reduced total porosity (Liu et al., 2022). Do sličnih zaključaka su došli i Martinez et al. (2011) navodeći da the highest soil moisture values at depths of up to 50 cm is in non tillage systems. Similar conclusions were reached by Martinez et al. (2011) stating that the highest soil moisture values at depths of up to 50 cm is in non tillage systems. That the tillage can significantly affect infiltration and evaporation in all soils and affects available water holding capacity and effective rooting depth in some soils was confirmed earlier by Subbulakshmi et al. (2009).

Results also suggest that management duration may not be the only factor that affects changes in soil hydraulic properties. Soil properties were assessed approximately one year after the last tillage, indicating the importance of temporal monitoring across seasons to comprehensively understand tillage effects. Variations in water infiltration and soil porosity between tillage systems are most pronounced immediately after tillage, diminishing over time due to soil consolidation (Strudley et al., 2008). While hydraulic properties like water infiltration and conductivity are highly impacted by tillage-induced changes in soil structure, water retention capacity remains less affected. Thus, ongoing monitoring and consideration of temporal dynamics are crucial for accurately characterizing tillage effects on soil hydraulic properties.

The various conclusions that emerged as a result of the research on the effects of land treatment and the impact of the treatment system on the flow of water and the characteristics of its retention in the soil justify the need for additional research.

CONCLUSION

Bearing in mind that the basic cultivation of the soil is an expensive and difficult agrotechnical measure that implies a large consumption of fuel per ha, a large amount of machine wear, that it affects the physical properties of the soil (positively and negatively), it should be given considerable attention. Both conventional and conservation tillage have their advantages and disadvantages. Therefore, it is necessary to observe land cultivation through a system of long-term trials, because anything other than that can lead to wrong conclusions. Today, when everything is viewed through economics, it should be borne in mind that tillage is an agrotechnical measure where rationalization is possible, but not significant savings. It is especially important to observe the cultivation through the crop rotation, and to practice rationalization and savings in the case of plant species that come second, third or later in the crop rotation, and special savings in cultivation can be achieved in the production of small grains.

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SECTION 5

RURAL DEVELOPMENT AND AGRO-ECONOMY

UDK: 634.85:631.15(497.2:4-672ЕУ)

CHANGES IN THE STRUCTURE OF BULGARIAN AGRICULTURAL HOLDINGS PRODUCING WINE GRAPES DURING THE YEARS OF EU MEMBERSHIP

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ABSTRACT

The tendencies in the development of viticulture and specifically the production of wine grapes in Bulgaria in the years after the country's accession to the EU were directly related to the changes in the structure of agricultural holdings growing vines with wine grape varieties. The processes of concentration and specialization of production largely determined the potential for sustainable development of the sector both nationally and regionally. The aim of the study was to identify the changes in the structure of the farms producing wine grapes from the point of view of the concentration of land resource and economic size. The official data from the censuses of agricultural holdings during the period 2007-2020 have been analysed. The ongoing structural changes in the wine sector in the years after the country accession to the EU lead to consolidation of the areas under vines in farms, with their average size per unit increased. The intensity of the changes in the regional plan was different, with average sizes of vineyard holdings producing wine grapes in the Southwest, Northwest and South-Central regions were smaller than the average for the country, and of the holdings in the Northeast, North Central and Southeast regions were a larger. Consolidation processes took place more intensively in the groups of holdings with a utilized agricultural area between 10 and 100 ha, which increased their percentage participation in the wine grapes production. The dynamics were more pronounced during the second application period of the Common agricultural policy.

Key words: *Vineyards, wine grapes, holdings, structural changes.*

INTRODUCTION

After Bulgaria's accession to the European Union, processes of change in the organizational and economic structure of Bulgarian agriculture have been taking place, with the main trends being related to reducing the number and consolidation of the average size of agricultural holdings (Koteva, 2014). Doitchinova et al. (2022) have pointed out that the permanent structural changes were seen in the concentration and specialization of production, the increase in the size of agricultural holdings and the annual liquidation of mainly small-sized holdings. According to the Adenauer-Stiftung (2015), cited by Popescu (2023), the general trend in the EU had been similar, with many small holdings ceasing their activities over the past two decades, "due to market pressure, the negative impact of climate change, the lack of financial resources to improve technologies and invest in innovations, the aging of farmers and the migration of young people to cities". Beluchova-Uzunova et al. (2023) confirmed this trend in Bulgaria for the period 2010-2020, indicating that the negative trend affected the rural areas development, where agriculture was largely the main activity for the population.

The operating production structures in viticulture were also influenced by the restructuring processes taking place in Bulgarian agriculture. Dimitrova and Dimitrov (2017a) studied the dynamics in the structure of farms growing vines, according to their number, average size, level of specialization and economic potential. They pointed out that the mechanisms of the National and Common Agricultural Policy during the first programming period of its application in our country did not significantly support the restructuring of the sector.

The development of grape production had been correlated with the organizational and economic conditions of combining production factors in the agricultural holdings (Dimitrova and Dimitrov, 2017b). According to Popescu (2023), the size of the holdings had been a key indicator in terms of resources, investments, production results and profitability. The agricultural land used by the holding was the most commonly applied and universal indicator when comparing the structure of agricultural holdings at the sectoral, national and international levels (Dimitrova, 2023; Popescu, 2023).

The objective of the study was to find out the change in the structure of agricultural holdings producing wine grapes, taking into consideration the changes in the concentration of areas planted with vineyards.

MATERIALS AND METHODS

The following indicators were used to study the structural changes (Koteva, 2014; Koteva et al., 2020; Koteva, 2023; Popescu, 2023):

Number of agricultural holdings producing wine grapes in total for the country and per statistical regions, NUTS 2 level of the European Classification of Territorial Units – Northwest, North Central, Northeast, Southeast, South Central and Southwest;

Change in the number of agricultural holdings in total for the country and per statistical regions during the study period (%);

Area of vineyards with wine grape varieties on agricultural holdings, in total for the country and per statistical regions (ha);

Change in the area of wine grape vineyards on agricultural holdings, in total for the country and per statistical regions during the study period (%);

Average size of the vineyard area for wine grape production in agricultural holdings, in total for the country and per statistical regions (ha);

Change in the average size of agricultural land used by the holdings during the study period (%).

The primary information was summarized based on the official data from the Ministry of Agriculture and Food (MAF), Agrostatistics Department from the censuses of agricultural holdings carried out in 2007, 2010, 2013, 2016 and 2020.

The changes in the number of agricultural holdings and the cultivated areas with wine grape vineyards were studied by farm size groups, categorized according to the classification used by Eurostat, and the Agrostatistics Department at the MAF, based on the size of the utilized agricultural area (UAA):

less than 2 ha of UAA;
from 2 to 5 ha of UAA;
from 5 to 10 ha of UAA;
from 10 to 20 ha of UAA;
from 20 to 30 ha of UAA;
from 30 to 50 ha of UAA;
from 50 to 100 ha of UAA;
over 100 ha of UAA.

The statistical processing of data on the number of holdings and areas in production units at the national level and per statistical regions was carried out using MS Excel. The methods of comparative analysis, structural analysis and descriptive statistics were applied (Petrov et al., 2004, Boshnakov, 2009, Shopova, 2018).

Variations in the studied indicators per years around their average values for the five studied years per statistical regions were determined using the coefficient of variation (Boshnakov, 2009, Li et al., 2016, Verdugo-Vásquez et al., 2021):

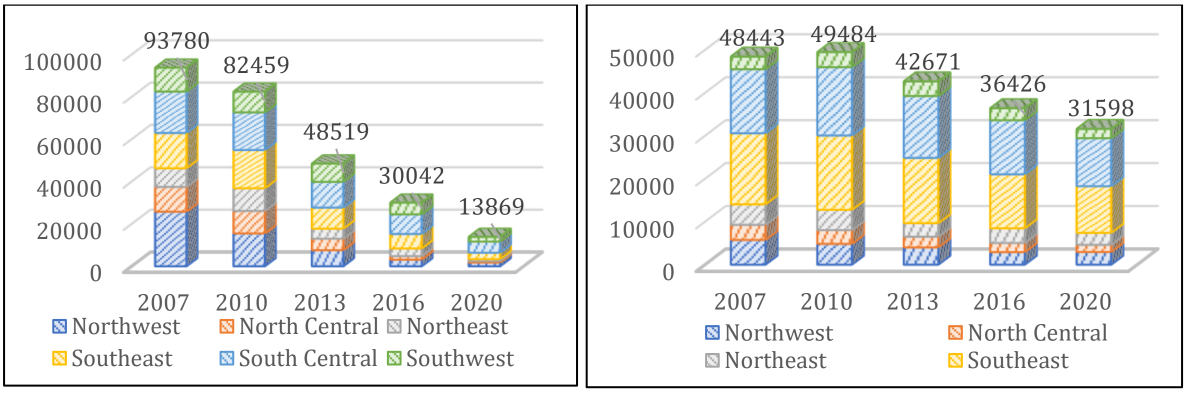
$$CV(\%) = \frac{SD}{\bar{Y}} * 100 = \frac{\sqrt{\frac{\sum_{i=1}^{n}(Y-\bar{Y})^2}{n}}}{\bar{Y}} * 100, \text{ where}$$

SD – standard deviation of the indicator (number of holdings and area of vineyards with wine grape varieties),

$\bar{Y}$ – average value of the indicator for the study period.

RESULTS AND DISCUSSION

The dynamics in the number of agricultural holdings had been an indicator that might be related both, to the economic viability of the wine sector and to its potential for attracting and retaining investor interest, for sustainable development of family businesses and for providing employment. According to Koteva (2023), the period following the country's accession to the EU was characterized by "dynamic structural changes" in agriculture, with one of the main trends being the significant decrease in the number of agricultural holdings – over 70% between 2007 and 2020. The trend observed in the wine sector was even more pronounced compared to the one outlined at the national level, as during the studied period the number of holdings producing wine grapes dropped from 93,780 to only 13,869, which represented a decrease of 85.2% (Fig. 1.A.). Examined per statistical regions, the negative dynamics in Northern Bulgaria were occurring at a faster pace than the national average, with the decrease in the number of agricultural holdings being the most significant in the North Central Region - by 95% in 2020 compared to 2007, followed by the Northwest and the Northeast Regions, respectively by 93.3% and 88.1%. In the three regions of Southern Bulgaria, the negative trend was developing at a slightly slower pace compared to the national average, with the decrease being relatively the smallest in the South-Central Region (by 73.1%), followed by the Southwest (by 80.2%) and the Southeast Regions (by 81.9%). The main reason for the more significant reduction in the number of wine grape producing holdings in Northern Bulgaria compared to the southern part of the country might be sought in the demographic processes, the negative manifestation of which was stronger in the three northern regions. The organizational characteristics of the production activity and economic results in wine holdings, primarily in terms of profitability, were other determinants. A similar conclusion was reached by Dimitrova (2023), according to which the decrease in the number of holdings in the country was related, on the one hand, to the consolidation of the agricultural units, on the other hand, the cause for this trend was the depopulation of rural areas and the reluctance of young people to engage in agriculture.



Source: Ministry of Agriculture and Food, Agrostatistics Department and own calculation

(A) number of holdings (B) vineyards areas in the holdings, ha

Figure 1. Dynamics of the number of holdings (A) and areas of vineyards with wine grape varieties (B) by statistical regions and total during the period 2007-2020

The decrease in the area of vineyards on farms occurred at a much slower pace than that of the number of farms, indicating ongoing processes of land consolidation in the agricultural holdings and a strengthening of their market orientation. The area of vineyards with wine grape varieties in the organizational structures operating in the sector decreased from 48.4 thousand ha in 2007 to 31.6 thousand ha in 2020 (Fig. 1.B.). The trend was the most pronounced again in the three regions of Northern Bulgaria, with the most significant decrease during the study period being recorded in the North Central Region (by 52.7%), followed by the Northwest (by 48.7%) and the Northeast (by 41.5%). In contrast, the negative changes in the cultivated areas were significantly milder in the South-Central Region, where the decline during the two compared years was 25%, as well as in the Southwest Region (27.1%) and in the Southeast Region (34.4%). Considered in absolute terms of the areas lost, the area of vineyards with wine grape varieties was the largest in the Southeast Region (reduction by 5,688 ha) and in the South-Central Region (reduction by 3,725 ha).

The coefficient of variation rates showed significant fluctuations in the number of holdings recorded across the five census years, around the average level for the studied period, which was due to the sharp drop of holdings in 2016 and 2020 (Table 1). The most intense changes were in the Northwest and North Central Regions with values of the indicator of 92.9% and 84.5%, respectively. Despite the strong reduction of the number of holdings in the South-Central Region, a lower degree of variation was found compared to the other five statistical regions with a coefficient value of 46.3%.

As far as the areas of wine vineyards in the holdings, slight deviations were observed per years compared to the average level for the period, with the values of the coefficient of variation ranging from 13.7% to 18.7% in the three regions of Southern Bulgaria, where the rates of decrease were slower, compared to 24.5% to 30.0% in the regions of Northern Bulgaria.

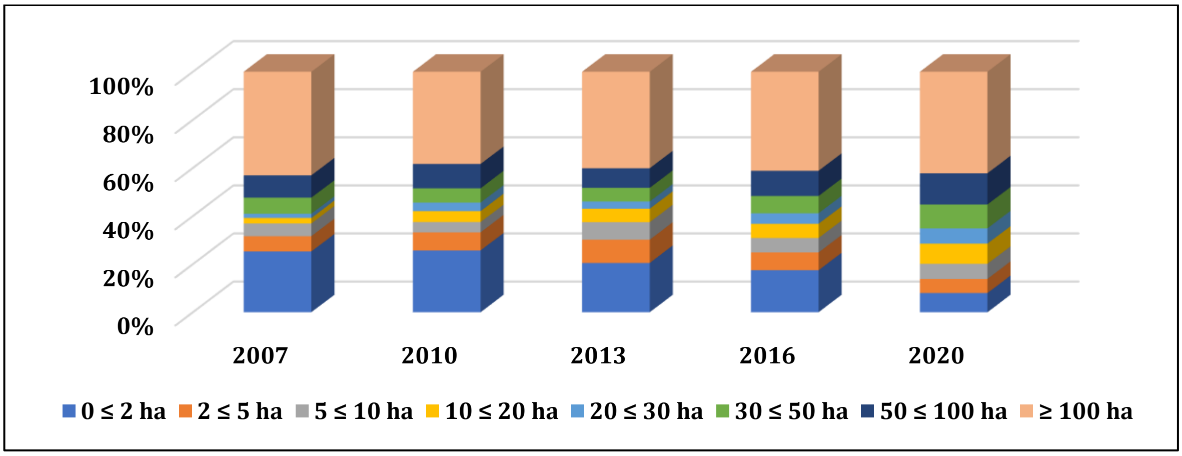
Table 1. Analysis of the variation of the number of holdings and cultivated area with wine grapes varieties by statistical regions

Number of holdings								
Statistical regions	**Indicators**							
	n	**R**	**Min**	**Max**	**Mean**	**Me**	**D**	**CV (%)**
Northwest region	5	24,046	1,735	25,781	10,741	7,565	9,977	92.9
North Central region	5	11,097	580	11,677	5,988	5,440	5,059	84.5
Northeast region	5	9,688	1,049	10,737	5,806	4,752	3,930	67.7
Southeast region	5	14,990	3,025	18,015	10,902	9,970	6,390	58.6
South Central region	5	14,306	5,254	19,560	12,801	12,111	5,926	46.3
Southwest region	5	9,039	2,226	11,265	7,496	8,681	3,625	48.4
Areas, ha								
Northwest region	5	2,842	2,905	5,747	4,086	4,005	1,226	30.0
North Central region	5	1,858	1,667	3,525	2,609	2,507	760	29.1
Northeast region	5	1,977	2,785	4,762	3,766	3,404	924	24.5
Southeast region	5	6,442	10,863	17,305	14,499	15,186	2,712	18.7
South Central region	5	4,721	11,198	15,919	13,814	14,434	1,895	13.7
Southwest region	5	1,352	2,139	3,491	2,949	2,935	542	184

Source: Ministry of Agriculture and Food, Agrostatistics Department and own calculations

The structuring of the land used for wine varieties vineyards in relation to the size of the utilized agricultural area (UAA) in the holdings, i.e. in the groups according to the physical size of the holdings had been of interest. The data presented in Fig. 2 revealed a certain polarization of the organization of cultivation the areas with wine grapes in the early years after our country's accession to the EU. In 2007, 25.3% of the vineyards area was cultivated in farms with a size of the utilized agricultural area of less than 2 ha, and 43.1% were in production structures with an UAA of over 100 ha. The ongoing structural processes, influenced by the mechanisms of the applied Common Agricultural Policy during both programming periods 2007-2013 and 2014-2020, changed the situation as in 2020 the share of the relatively small agricultural land used by the holdings had dropped down significantly, with 6.9% of the total area of wine vineyards in the production units remaining in them. In this group, a severe reduction in the cultivated areas was reached – by 79.4% in 2020 compared to 2007. The observed dynamics of restructuring in the wine sector corresponded to the ongoing trends in agricultural holdings in Bulgaria in general, with Koteva (2023) indicating that "the reduction processes were the most dynamic in holdings with an UAA of up to 2 ha, where the decrease was by 84%. The share of holdings with an UAA between 2 and 5 ha was also decreasing – from 6.4% in 2007 to 5.1% in 2020, where the reduction of the cultivated areas was 39.4%.

Regardless the slower decrease in areas compared to the number of holdings indicated an increase in the agricultural land used by them, the drop in the area of wine vineyards in the agricultural holdings with an UAA of over 100 ha might be considered an unfavourable fact – from 20.8 thousand ha in 2007 to 13.3 thousand ha in 2020 (reduced by 35.9%). According to Harizanova (2015), cited by Dimitrova (2023), large holdings had more opportunities to diversify risk, which was a key point in organizing and managing activities in the wine sector, characterized by a high degree of risk, due to the significant amount of investment in creating vineyard plantations and the long term of their exploitation. The ratio of vineyards area for wine grapes production in this group of holdings shrunk to 36.6%.



Source: Eurostat (*https://ec.europa.eu/eurostat*) and own calculations

Figure 2. Change in the percentage of the wine vineyards area in agricultural holdings in Bulgaria per groups, according to the size of the utilized agricultural area (UAA)

Positive dynamics were observed in the groups of agricultural holdings with UAA between 10 and 20 ha and between 20 and 30 ha, which expanded their percentage share of the total area of cultivated vineyards with wine grape varieties, respectively from 2.3% and 1.8% in 2007 to 7.3% and 5.5% in 2020. In these two groups, the size of cultivated areas steadily increased – by 1,550 ha in the group with UAA between 10 and 20 ha and by 1,160 ha in the group with UAA between 20 and 30 ha. During the period covered by the study, the percentage of agricultural holdings with UAA between 30 and 50 ha and between 50 and 100 ha was also expanding, respectively 8.6% and 11.3% of the area of wine grape vineyards in the country. The difference in this case was that in both groups there was a decrease in the cultivated areas, but to a much lesser extent than reported at the national level and in the already mentioned groups with a marked reduction in the areas.

Although the ongoing processes of restructuring the agricultural holdings at the regional level followed the general trend, observed for the national level, they had their own specificity, which largely substantiated the current state of the sector and the prospects for its future development.

The structural processes in wine grape production, both in the country as a whole and in the Northwest Region, were taking place more strongly during the second period of application of the CAP. The data in Table 2 demonstrated that the number of holdings was decreasing at a faster pace in the years after 2013, as it referred mainly to agricultural units with a small UAA size – up to 5 ha.

The decrease in the number of holdings using small agricultural land in the North Central Region was even more severe. The number of agricultural units with an UAA of up to 2 ha decreased by 96.6% in 2020 compared to 2007, while that of holdings with an UAA between 2 and 5 ha – by 93.3%. The negative dynamics were slightly less pronounced for larger holdings, with the decrease in structures with an UAA between 30 and 50 ha being 42.9%, and in those with an UAA between 50 and 100 ha – by 33.3%. They constituted 6.9% and 3.4% of the holdings operating in the region in 2020, respectively, but concentrated 10.2% and 21.6% of the total area of vineyards with wine grape varieties.

The general trend of change outlined in the Northeast Region showed a reduction in the number of holdings growing vineyards for wine grape production by 88.1%, with it being the most significant in the groups of production structures with an UAA of up to 2 ha and between 2 and 5 ha, by 89.8% and 89%, respectively.

Unlike the regions in North Bulgaria, in the Southeast region – one of both main regions where the area of vineyards with wine grape varieties in the country was concentrated, the trend of decreasing the number of agricultural holdings did not cover all groups of farms. The reduction in the number of agricultural holdings was strongly expressed in the holdings with a smaller size of the available land resource in the groups with an UAA of up to 20 ha, while the number of the largest holdings – with an UAA of over 100 ha – was also dropping down.

Despite the certain fluctuation per years, in 2020 compared to 2007, the number of holdings in the groups with an UAA between 20 and 30 ha, between 30 and 50 ha and between 50 and 100 ha went up. That was evidence for the presence of entrepreneurial interest and the opportunities for investing capital in the sector that existed at the regional level.

Similar structural processes were taking place in the second leading region in the country’s wine sector – South Central. The total decrease in the number of the agricultural holdings for the entire fourteen-year period of the study was 73.2%, but that trend did not cover all groups of farms. The reduction, as in the other statistical regions, was the most extreme in farms with a smaller size of the utilized agricultural areas – up to 10 ha. In all other groups, the number of agricultural holdings located in the region was increasing compared to 2007.

The restructuring of farms in the Southwest region was also more intense during the second period of application of the CAP. Considered for the entire period 2007-2020, the number of agricultural holdings producing wine grapes with a small UAA size - up to 2 ha and from 2 to 5 ha - decreased the most, with the reduction in the two groups being by 86% and by 45.8%, respectively. Regardless of the fluctuations in individual years, the number of holdings in all other groups increased:

by 110 holdings in the group with an UAA between 5 and 10 ha;
by 90 holdings in the group with an UAA between 10 and 20 ha;
by 50 holdings in the group with an UAA between 20 and 30 ha;
by 70 holdings in the group with an UAA between 30 and 50 ha;
by 40 holdings in the group with an UAA between 50 and 100 ha;
by 40 holdings in the group with over 100 ha of UAA.

Table 2. Distribution of the number of farms growing vineyards with wine grape varieties, according to the size of the UAA per statistical regions (NUTS 2) during the period 2007-2020

Indicators	**Number of holdings per group of utilized agricultural area**							
Northwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	22,970	1,720	500	250	70	80	80	110
2010	13,650	1,090	340	190	80	60	50	60
2013	6,200	770	260	140	70	50	30	70
2016	2,390	360	160	80	40	30	20	40
2020	1,160	230	110	130	40	40	30	40
2020/2007 %	5.1	13.4	22.0	52.0	57.1	50.0	37.5	36.4
Northcentral region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	9,570	1,200	380	210	10	70	30	110
2010	8,900	890	370	190	70	60	60	70
2013	4,240	520	370	10	50	10	70	60
2016	1,340	50	60	70	30	20	10	30
2020	330	80	50	30	20	40	20	40
2020/2007 %	3.4	6.7	13.2	14.3	18.2	57.1	66.7	36.4
Northeast region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	7,460	910	170	110	50	30	60	40
2010	9,710	450	220	160	60	50	40	50
2013	3,870	400	160	100	90	40	20	50
2016	3,100	240	110	100	30	20	30	40
2020	760	100	50	40	30	20	20	30
2020/2007 %	10.2	11.0	29.4	36.4	60.0	66.7	33.3	75.0
Southeast region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	13,740	1,540	520	410	60	110	90	200
2010	15,280	1,170	560	370	160	130	150	200
2013	7,800	890	520	310	80	160	80	170
2016	4,950	750	410	270	100	120	70	180
2020	1,470	450	320	240	130	140	100	180
2020/2007 %	10.7	29.2	61.5	58.5	216.7	127.3	111.1	90.0
Southcentral region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	17,150	1,570	520	80	50	60	40	90
2010	15,340	1,450	440	220	80	80	80	110
2013	9,670	1,390	420	230	140	90	70	110
2016	7,500	880	320	100	140	170	50	160
2020	3,420	710	350	240	120	160	110	140
2020/2007 %	19.9	45.2	67.3	300.0	240.0	266.7	275.0	155.6
Southwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	10,720	480	10	20	0	10	20	0
2010	9,170	450	60	50	20	20	20	10
2013	7,480	550	390	90	60	60	30	10
2016	4,850	370	110	60	40	50	10	20
2020	1,500	260	120	110	50	80	60	40
2020/2007 %	14.0	54.2	1200.0	550.0	x	800.0	300.0	x

Source: Eurostat (https://ec.europa.eu/eurostat) and own calculations

In all six statistical regions of Bulgaria, divided according to NUTS 2, the decrease in the area of vineyards planted with wine grapes varieties was occurring at a slower rate compared to the determined drop down in the number of agricultural holdings (Table 3).

The reduction in the Northwest Region was by 2,160 ha in 2013 compared to 2007 (-37.6%), and by 640 ha in 2020 compared to 2013 (-17.8%). The slower reduction rate of the areas led to an increase in the average area per holding, with the most significant growth in the used agricultural land in organizational holdings with an UAA between 20 and 30 ha – from 0.86 ha to 6.0 ha during the study period (Table 4).

The decrease in the area of vine plantations grown for wine grape production in the North Central Region was more significant than in the Northwest Region – by 52.7% during the fourteen years of the study period. The agricultural land planted with vineyards for wine grape production went down in all groups of farms, with the reduction being the most significant in the small-sized ones with an UAA of up to 2 ha – by 93.9%. The average area of vineyards in one agricultural unit in the groups of large holdings with an UAA between 50 and 100 ha and over 100 ha had very close values, 18 ha and 19.25 ha respectively, due to the different direction of the dynamics of the cultivated areas. What was common between both groups was the reduction in areas during the first period of application of the CAP until 2013. Thereafter, an increase in hectares of wine vineyards was observed in both groups of farms by 2016, however in the next four years that trend continued only in the agricultural holdings with an UAA between 50 and 100 ha.

The situation was different in the Northeast Region, where the average size of the areas with vineyards in the groups of large holdings exceeded that rate for the other regions (Table 4). The main part of the area (76.6%) in 2020 was concentrated in three groups: agricultural holdings with an UAA of over 100 ha cultivated 43.9% of the total vineyards area for the region; with an UAA between 50 ha and 100 ha – 18.0%; with an UAA between 30 and 50 ha – 14.7%. The participation of small agricultural holdings with an UAA of up to 5 ha was gradually decreasing, with the areas cultivated by them at the beginning of the period represented 24.6% of the area of vineyards, and in 2020 – it shrank to 8.6%. Some of the vineyards were most likely abandoned, but another part was consolidated in the agricultural holdings.

Table 3. Distribution of areas with vineyards for wine grape production in agricultural holdings, according to the size of the UAA per statistical regions (NUTS 2) during the period 2007-2020

Indicators	**Area of vineyards with wine grape varieties, ha**							
Northwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	1,900	240	120	80	60	220	290	2,840
2010	1,430	290	190	230	220	310	250	1,910
2013	660	290	130	330	50	210	160	1,760
2016	320	120	200	240	170	240	610	1,000
2020	240	110	130	220	240	400	350	1,260
2020/2007, %	12.6	45.8	108.3	275.0	400.0	181.8	120.7	44.4
North Central region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	660	90	220	110	290	220	420	1,520
2010	660	180	140	140	190	250	270	1,360
2013	330	150	300	100	200	90	200	890
2016	180	120	80	130	70	230	310	1,030
2020	40	50	80	100	100	170	360	770
2020/2007, %	6.1	55.6	36.4	90.9	34.5	77.3	85.7	50.7
Northeast	0 ≤ 2	2 ≤ 5	5 ≤ 10	10 ≤ 20	20 ≤ 30	30 ≤ 50	50 ≤ 100	≥ 100

region	ha	ha	ha	ha	ha	ha	ha	ha
2007	810	360	200	30	70	230	680	2,380
2010	1,180	200	160	190	140	360	910	1,600
2013	570	110	90	130	50	280	70	1,400
2016	380	190	80	140	320	370	630	1,290
2020	150	90	90	150	170	410	500	1,220
2020/2007, %	18.5	25.0	45.0	500.0	242.9	178.3	73.5	51.3
Southeast region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	2,860	960	1,280	650	230	510	1,950	8,110
2010	3,370	1,150	800	810	640	960	1,820	7,750
2013	2,130	1,150	890	800	370	970	1,460	7,350
2016	1,640	830	730	850	320	900	1,090	6,220
2020	500	540	660	970	640	920	1,140	5,480
2020/2007, %	17.5	56.3	51.6	149.2	278.3	180.4	58.5	67.6
South Central region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	3,770	1,120	690	190	200	1,880	1,110	5,960
2010	4,020	1,280	680	650	400	870	1,720	6,300
2013	3,260	1,770	1,080	620	490	670	1,380	5,170
2016	2,610	1,140	840	590	610	740	1,150	4,900
2020	1,160	800	820	940	610	960	1,420	4,490
2020/2007, %	30.8	71.4	118.8	494.7	305.0	51.1	127.9	75.3
Southwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	2,220	300	40	40	0	170	50	0
2010	2,020	620	140	210	170	180	20	0
2013	1,520	520	520	320	90	120	100	0
2016	1,230	290	260	200	110	130	30	550
2020	430	270	200	270	250	270	340	110
2020/2007, %	19.4	90.0	500.0	675.0	x	158.8	680.0	x

Source: Eurostat (https://ec.europa.eu/eurostat) and own calculations

In the Southeast Region, the areas with vineyards for wine grape production increased only in the groups with an UAA between 20 and 30 ha and between 30 and 50 ha, by 410 ha in both cases. In contrast, in large holdings, the areas decreased, which also affected the level of concentration of the used land resource in them. In terms of the cultivated area structure, throughout the entire period, the major place in the management of the land resource in viticulture was taken by large agricultural holdings with an UAA over 100 ha, with their share in 2020 being 50.5%. The percentage share of holdings with an UAA between 50 and 100 ha decreased slightly to 10.5%. The share of small farms with an UAA of less than 2 ha was also dropping down (from 17.3% to 4.6%), from 2 to 5 ha (from 5.8% to 5.0%) and from 5 to 10 ha (from 7.7% to 6.1%). A more pronounced positive dynamics was observed in holdings with an UAA between 10 and 20 ha, as the areas cultivated by them represented 1.4% in 2007, but the ratio of this group in the total area went up to 8.9% in 2020. The share of the agricultural structures with an UAA between 20 and 30 ha increased slightly – from 1.4% to 5.9%, and the percentage of holdings with an UAA of between 20 and 30 ha raised to 8.5%.

In the South-Central Region, the total area of vineyards with wine grape varieties shrank by 24.9%, and similarly to the Southeast Region, the rate of decrease was more pronounced during the second period of the CAP application, when 3,240 ha of wine vineyards remained outside the holdings, compared to 480 ha during the years from 2007 to 2013. Considered by farm groups, the dynamics were not similar, as during the period 2007-2020, the areas of vineyards decreased in the holdings with an UAA of up to 2 ha (-69.2%), from 2 to 5 ha (-28.6%), from 30 to 50 ha (-48.9%) and over 100 ha (-24.7%). In the remaining groups, the area of vineyards went up as the increase was the most significant in farms with an UAA between 10 and 20 ha – by 750 ha, followed by farms with an UAA between 20 and 30 ha – by 410 ha, farms with an UAA from 50 to 100 ha – by 310 ha and farms with an UAA between 5 and 10 ha – by 130 ha. The increased number of holdings was the reason for the reducing the average size of the cultivated areas with vineyards in one agricultural production structure, which was observed in holdings with an UAA over 30 ha (Table 4). The average area of vineyards for wine grape production in large holdings with an UAA between 50 and 100 ha had been reduced by more than twofold – from 27.75 ha in 2007 to 12.91 ha in 2020, as well as in holdings with an UAA over 100 ha – from 66.22 ha to 32.07 ha.

The decrease was even more noticeable in agricultural holdings with an UAA of 30 to 50 ha – from 31.33 ha at the beginning of the period to 6.0 ha at the end. The data indicated a change in the specialization of the agricultural structures having significant available land resources that occurred under the influence of both the market environment and the national and sectoral agricultural policy.

In the structure of the total area of vineyards in the holdings in the region, the largest share was held by farms with an UAA of over 100 ha, which remained almost constant during the individual years of the censuses – about 40%. The percentage share of agricultural units with an UAA between 50 and 100 ha was expanding – from 7.4% to 12.7%, with an UAA between 20 and 30 ha – from 1.3% to 5.4%, with an UAA between 10 and 20 ha – from 1.3% to 8.4% and with an UAA between 5 and 10 ha – from 4.6% to 7.3%. The percentage of small farms with an UAA up to 2 ha was decreasing – from 25.3% to 10.4% and with an UAA between 2 and 5 ha – from 7.5% to 7.1%. The trends showed a goal to maintain the viability of wine grape and wine production in agricultural units that do not have large land resources, and probably also financial resources for a more serious expansion of the business. Some of these holdings were family wineries that offer a diverse product range of boutique wines and sell their production in specific market niches.

In the Southwest Region, the distribution of the area of vineyards with wine grape varieties in the groups of holdings with an UAA between 2 and 50 ha was almost even, respectively: 12.6% in farms with an UAA between 2 and 5 ha; 9.3% in farms with an UAA between 5 and 10 ha; 12.6% in farms with an UAA between 10 and 20 ha; 11.7 in farms with an UAA between 10 and 20 ha; 12.6% in farms with an UAA between 30 and 50 ha. The data revealed that the structural processes in the region were related to the increase in the market orientation of a large part of the agricultural holdings and the aim for sustainable business development in the sector. A distinctive feature of the structural changes in the Southwest Region was the accounted increase in the areas of the holdings during the years from 2007 to 2013 – by 8.9%.

Table 4. Average size of areas with vineyards for wine grape production in agricultural holdings, according to the size of the UAA per statistical regions (NUTS 2) during the period 2007-2020

Indicators	**Average size of areas with vineyards for wine grape production in agricultural holdings, ha**							
Northwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	0.08	0.14	0.24	0.32	0.86	2.75	3.63	25.82
2010	0.10	0.27	0.56	1.21	2.75	5.17	5.00	31.80
2013	0.11	0.38	0.50	2.36	0.71	4.20	5.33	25.14
2016	0.13	0.33	1.25	3.00	4.25	8.00	30.50	25.00
2020	0.21	0.48	1.18	1.69	6.00	10.00	11.67	31.50
2020/2007, %	262.5	342.9	491.7	528.1	697.7	363.6	321.5	122.0
North Central region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha

2007	0.07	0.08	0.58	0.52	2.64	3.14	14.00	13.82
2010	0.07	0.20	0.38	0.74	2.71	4.17	4.50	19.43
2013	0.08	0.29	0.81	0.91	4.00	9.00	2.86	14.83
2016	0.13	2.40	1.33	1.86	2.33	11.50	31.00	34.33
2020	0.12	0.63	1.60	3.33	5.00	4.25	18.00	19.25
2020/2007, %	173.2	781.3	275.9	641.0	189.4	135.4	128.6	139.3
Northeast region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	0.11	0.40	1.18	0.27	1.40	7.67	11.33	59.50
2010	0.12	0.44	0.73	1.19	2.33	7.20	22.75	32.00
2013	0.15	0.28	0.56	1.30	0.56	7.00	3.50	28.00
2016	0.12	0.79	0.73	1.40	10.67	18.50	21.00	32.25
2020	0.20	0.90	1.80	3.75	5.67	20.50	25.00	40.67
2020/2007, %	179.4	225.0	152.5	1388.9	404.8	267.3	220.7	68.3
Southeast region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	0.21	0.62	2.46	1.59	3.83	4.64	21.67	40.55
2010	0.22	0.98	1.43	2.19	4.00	7.38	12.13	38.75
2013	0.27	1.29	1.71	2.58	4.63	6.06	18.25	43.24
2016	0.33	1.11	1.78	3.15	3.20	7.50	15.57	34.56
2020	0.34	1.20	2.06	4.04	4.92	6.57	11.40	30.44
2020/2007, %	162.0	193.5	83.8	254.2	128.5	141.6	52.6	75.1
South Central region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	0.22	0.71	1.33	2.38	4.00	31.33	27.75	66.22
2010	0.26	0.88	1.55	2.95	5.00	10.88	21.50	57.27
2013	0.34	1.27	2.57	2.70	3.50	7.44	19.71	47.00
2016	0.35	1.30	2,63	5.90	4.36	4.35	23.00	30.63
2020	0.34	1.13	2,34	3.92	5.08	6.00	12.91	32.07
2020/2007, %	154,2	158,7	176,2	164.6	127.1	19.2	46.5	48.4
Southwest region	0 ≤ 2 ha	2 ≤ 5 ha	5 ≤ 10 ha	10 ≤ 20 ha	20 ≤ 30 ha	30 ≤ 50 ha	50 ≤ 100 ha	≥ 100 ha
2007	0.21	0.63	4,00	2.00	0.00	17.0	2.5	0.0
2010	0.22	1.38	2,33	4.20	8.50	9.0	1.0	0.0
2013	0.20	0.95	1,33	3.56	1.50	2.0	3.3	0.0
2016	0.25	0.78	2,36	3.33	2.75	2.6	3.0	27.5
2020	0.29	1.04	1,67	2.45	5.00	3.38	5.7	2.8
2020/2007, %	136.5	164.8	41.7	122.7	x	19.9	226.7	x

Source: Eurostat (https://ec.europa.eu/eurostat) and own calculations

The trend was evident in the groups with UAA between 2 and 5 ha (by 220 ha), between UAA 5 and 10 ha UAA (by 480 ha), between UAA 10 and 20 ha (by 280 ha), between UAA 20 and 30 ha (by 90 ha) and between UAA 50 and 100 ha (by 50 ha). So far, there were no wine vineyards in the farms with the largest UAA size over 100 ha.

The data clearly showed the consolidation of the agricultural structures, that happened based on the reduction of areas in the holdings with the smallest UAA size up to 2 ha, as well as the presence of entrepreneurial interest and implemented investments in planting new wine vineyards. The unfavourable fact was that this dynamic was not sustainable and in the following years, the areas of vineyards with wine varieties in the region had decreased. The reduction was 1,050 ha in 2020 compared to 2013 and was mainly a result of the decline recorded in the smallest farms with an UAA of up to 2 ha – by 1,090 ha. A decrease was also observed in the areas in the groups of holdings with an UAA between 2 and 20 ha, while in the larger structures in terms of used agricultural land for growing wine vineyards there was an increase as follows: by 160 ha in the farms in the group with an UAA between 20 and 30 ha; by 150 ha in the farms in the group with an UAA between 30 and 50 ha; by 240 ha in the farms in the group with an UAA between 50 and 100 ha; with 110 ha in the farms in the group with UAA over 100 ha.

Despite the positive dynamics, the concentration of vineyard areas within a separate agricultural unit in the Southwest Region happened the slowest compared to the trend described in the other statistical regions. The average size of the areas with wine grape varieties was the smallest, both in the region as a whole (0.96 ha compared to 2.28 ha on the average for the country), and in the individual groups of holdings with a relatively large UAA size of over 30 ha. At the same time, in this region the percentage of the areas of vineyards with wine varieties in small UAA farms up to 5 ha was the most significant (32.7%), which was a prerequisite for restructuring to continue in the coming years. A similar prognosis, but with regard to the development of the organizational and economic structure of agriculture at the national level, was made by Koteva (2023). According to her "the trend of reducing the number of holdings, mainly at the expense of the small ones, was expected to continue, but at a slower rate".

Although, due to structural changes, the average size of the areas with vineyards for wine grapes production in agricultural holdings in Bulgaria increased to 2.28 ha in 2020, it had remained significantly below the average size of the arable areas in agriculture at the national level – 33 ha (Koteva, 2023). That was found in almost all groups of agricultural structures with different sizes of UAA, as for the smallest farms with UAA up to 2 ha, the average size of the areas per statistical regions varied from 0.12 to 0.34 ha with an average for the country of 0.6 ha. There was an extreme difference between the agricultural land used in the groups of large holdings with UAA over 50 ha, where the average value at the national level was 320.6 ha according to data from Koteva (2023). In the wine sector, the largest areas were in the biggest holdings with an UAA of over 100 ha, which in the individual regions, except the Southwest one, were between 19.25 ha and 40.67 ha. According to Koteva (2023), the consolidation of areas in the agricultural holdings was accelerated mainly under the impact of direct payments. This dynamics was more intense in the grain and oilseed sectors, while in viticulture, the payments per area did not play the role of a main incentive for speeding up structural changes. In the sector, the role of the market and institutional environment was important, as well as the specifics of the regional conditions and the opportunities for diversifying profitability and risk.

The agricultural land used by the holdings had been an important indicator, but in itself it did not provide sufficiently detailed information about the restructuring processes. Very often, this classification indicator was considered simultaneously with other indicators that more accurately outlined the picture of production structures and their characteristics (Dimitrova, 2023). With a view of this, the analytical work related to the development of the organizational and economic structure of wine viticulture in Bulgaria continued.

CONCLUSION

The ongoing structural changes in the wine sector after our country's accession to the EU had led to the consolidation of the areas with wine vineyards in the holdings, with their average size in one agricultural unit increasing from 0.52 ha in 2007 to 2.28 ha in 2020. The intensity of the changes in regional terms was different, with holdings in the Southwest (0.96 ha), Northwest (1.66 ha) and South Central (2.13 ha) Regions having a smaller average size of the areas than the average for the country, and holdings in the Northeast (2.66 ha), North Central (2.88 ha) and Southeast Regions (3.58 ha) having a larger one.

The number of holdings was reduced more significantly than the decrease in the size of the cultivated areas. Considered per statistical regions, the reduction in the number of the agricultural units was more significant in the three statistical regions of Northern Bulgaria, due to the more pronounced negative dynamics of demographic processes, as well as problems of a technological and organizational nature, leading to unsatisfactory economic results.

In the three regions of South Bulgaria, the negative trend was developing at a slower pace compared to the dynamics at the national level, with the number of holdings decreasing the slowest in the South Central Region, followed by the Southwest and the Southeast Regions. The rates of decrease in the areas with wine vineyards in the holdings in these three regions were also slower compared to the regions of North Bulgaria. The larger number of holdings operating in the same agricultural sector at the local and regional level, in addition to driving the competitive environment, had created wider opportunities for cooperation, building network structures or integrating horizontally and vertically in the value chain.

The restructuring processes, influenced by the mechanisms of the Common Agricultural Policy implemented during the two programming periods 2007-2013 and 2014-2020, were changing the structure of the areas with wine vineyards per groups of holdings with different sizes of the utilized agricultural area. The percentage of the areas in the smallest holdings using agricultural land was shrinking severely from 25.3% in 2007 to 6.9% in 2020. The share of the largest agricultural structures with an UAA of over 100 ha was also decreasing – from 43.1% to 36.6%, which was indicative that the implemented subsidy policy in agriculture stimulated the orientation towards the cultivation of crops with lower production costs.

The consolidation processes were more intense in the groups with UAA between 10 and 100 ha, which increased their participation in the production activity in the sector. The dynamics had been more pronounced during the second period of application of the CAP.

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THE DEGREE OF INCOME DIVERSIFICATION AMONG RURAL HOUSEHOLDS IN NORTH MACEDONIA: A CASE STUDY OF POLOG AND PELAGONIA REGIONS

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ABSTRACT

Traditionally, rural development was closely linked to agricultural growth; however, modern strategies emphasize the importance of non-farm rural employment (NFRE) for enhancing rural livelihoods. This study examines income diversification in rural households in North Macedonia, with a focus on the Polog and Pelagonia regions. This research employs the Shannon Equitability Index (E) to measure the degree of income diversification across various household income sources. Derived from the Shannon Index, the Equitability Index considers both the number and equity of income sources, offering a comprehensive perspective on income distribution within households. The index ranges from 0 to 100, where higher values indicate greater income diversification.

Data for this study were collected through field interviews with 140 rural households in Pelagonia and Polog in 2018, categorizing income into five groups: crop production, livestock production, non-agricultural activities, off-household income and transfers. Households were classified based on their poverty status, distinguishing those above and below the poverty line. Findings reveal low income diversification levels in both regions, with Shannon Index values of 32.9 for Pelagonia and 35.2 for Polog. Notably, households below the poverty line tend to have a more equitable income distribution among various sources than wealthier households. These results highlight the tendency of poorer households to distribute their income sources more evenly, contrasting with wealthier households that show greater reliance on specific income streams. This study thus contributes to a better understanding of income diversification's role in rural development and poverty alleviation in North Macedonia.

***Key words**: Shannon Index, Polog, Pelagonija, Equitability, Rural households.*

INTRODUCTION

Rural livelihood diversification

Family farms face serious economic, political, social, and environmental challenges. In the mid-20th century, when economic theory was dominated by industrialization as the leading model for economic development, the rural economy was considered a low-productivity sector supplying inferior and less important products to the market (Bogdanov, 2015). The deterioration of trade conditions due to globalization and increased competition, rural-urban migration, declining population density in rural areas, reduced availability of natural resources, and other factors present a bleak outlook for small family farms relying solely on agricultural activities (Kovachevikj, 2013). To ensure farm continuity and long-term survival, agricultural families increasingly adopt market-driven, innovative, and sustainable plans, recognizing that agriculture and food production are no longer the sole functions of rural areas (Suess-Reyes & Fuetsch, 2016). Rural populations are compelled to seek alternative livelihood strategies to reduce their dependence on agriculture as the sole income source (Kovachevikj, 2013). Household income strategy can encompass a wide range of other diversified activities aimed at meeting the needs of the rural population: the production of specialty foods characterized by traditional flavors, the collection of medicinal, aromatic, and ornamental plants, rural tourism, the valorization of natural assets and the traditional appearance of rural communities, craftsmanship with a focus on handmade products and artisanal services, and more (Kovachevikj, 2013).

Rural economic diversification is critical for sustainable resource management and household welfare in rural areas. Defined as the production of diverse goods and services within a rural household's domain (Mehta, 2009), diversification enables households to generate income from multiple sources, mitigating risks associated with reliance on a single activity. While driven primarily by economic factors, non-economic considerations may also influence diversification strategies (ibid). Over the past two decades, shifts in agricultural demand towards new rural services and products and declining farm incomes have forced rural households to be engaged in both on-farm and off-farm activities (Haines & Davies, 1987; Rantamäki-Lahtinen et al., 2005). Such diversification has significant socioeconomic and environmental implications, extending beyond rural communities (Dharmawan & Manig, 2000). Empirical studies highlight the link between rural poverty and limited access to land, livestock, as well as the inability to secure alternative income sources unrelated to agriculture (Ellis & Bahigwa, 2001). High poverty rates and uneven development in rural areas, coupled with intensive urbanization, have increased focus on the rural non-farm economy (RNFE) as an intersectoral link between urban industry and rural agricultural economy (Bogdanov, 2015). Diversification, whether through non-agricultural ventures or rural non-farm industries, is widely recognized as an effective poverty-reduction strategy (Reardon, 1997; Haggblade et al., 1989).

Scholars recognize two distinct conceptual approaches to defining rural diversification:

1. The multifunctionality concept – encompassing both on-farm and off-farm activities such as agricultural production, agritourism, machinery rentals, primary product processing, renewable energy generation, and similar activities (Start & Johnson, 2001).
2. The non-agricultural linkages concept – covering only activities unrelated to primary agricultural production but directly or indirectly connected to resources of the family farms, including hospitality, rural tourism, sports, cultural and recreational activities, handicrafts, aquaculture, wild plant gathering, etc. (*ibid*).

This research adopts the approach defined by the European Commission (2008), which describes rural diversification as the development of income-generating activities that are not directly related to agricultural production but are closely connected to rural households' resources or enterprises. These activities involve the use of available resources or products that have an economic impact on the household or farm (EC, 2008). Diversification is not new to farmers, since the agricultural history traces such practices back to the Middle Ages (Friedmann, 1986). However, the importance of diversification has grown significantly over the past 15–20 years. As of the latest available data, approximately one in three farms in the European Union are engaged in diversified activities beyond traditional agriculture (European

Court of Auditors, 2022). Alternative income sources are more prevalent in Western and Northern Europe (e.g., France, the UK, Germany) than in Eastern and Southern Europe.

Income diversification affects households' overall welfare. The level and type of income diversification in rural households depends on the availability of different income sources and how different household types respond to them, which may in turn depend on geographic location, access to labor markets and factories, human and social capital, and periodic policy changes. Effective resource management requires rural households to develop business strategies demanding management and marketing skills. The chosen combination of on-farm activities will determine the household's overall welfare. When evaluating alternatives, the feasibility and efficiency of resource use must be prioritized (Suess-Reyes & Fuetsch, 2016).

Empirical studies show that education and infrastructure access are strong determinants of diversification (Barrett et al., 2001; Block & Webb, 2001).

Considering the circumstances of RNFE development, economic diversification in rural areas solves numerous problems:

- Absorbs surplus rural labor and reduces hidden unemployment;
- Reduces risk for farm households through activities complementing or replacing agricultural income;
- Provides household survival when agricultural production is destroyed or threatened by adverse weather and other risks;
- Enhances and efficiently utilizes rural areas' comparative advantages (natural/physical resources, low labor costs etc.);
- Contributes to faster economic growth in rural areas;
- Improves overall quality of life and increases availability for diversification of products and services in rural areas;
- Reduces rural migration because some rural non-agricultural activities are much more attractive to young people than agricultural production, such as rural tourism (Bogdanov, 2015).

Literature presents various classifications of rural economic diversification. Scientific understanding of diversification models and types remains conceptually incomplete. Most approaches consider socioeconomic status of households and local economic environment. Classification can follow various criteria. One widely framework is provided by Davis and Pearce (2001), who classify income diversification based on the underlying motivation for engaging in non-farm activities. Their typology distinguishes between two primary path ways:

1) Poverty-driven ("distress-push"), and
2) Demand-driven ("demand-pull") (Table 1).

Table 1. Push-Pull factors of rural livelihood diversification

Distress push factors	Demand pull factors
▪ Population growth. ▪ Limited access to fertile land. ▪ Low farm productivity. ▪ Low agricultural yields. ▪ Lack of access to agricultural markets. ▪ Declining access to natural resources. ▪ Temporary shocks and crises. ▪ Limited access to rural financial markets.	▪ Higher returns on labor from RNFE activities. ▪ Higher return on investment in RNFE. ▪ Lower risk in RNFE compared to agricultural activities. ▪ Cash generation to meet household needs. ▪ Availability of economic opportunities and social benefits in and beyond rural areas. ▪ Urban population's dissatisfaction with city life.

(Davis & Pearce, 2001)

Distress-push diversification typically arises in contexts marked by risk, market imperfections, and hidden agricultural unemployment. It is often a reaction to economic hardship, such as population pressure, limited access to productive land, low agricultural yields, and poor market access, which forces households into non-farm activities to prevent further income decline. These activities are usually less productive than agriculture would be under conditions of full employment, but are pursued as a survival strategy.

In contrast, demand-pull diversification is driven by emerging market or technological opportunities that make non-farm rural employment (RNFE) more attractive. It reflects proactive choices by households seeking to increase returns on labor and investment, reduce economic risk, or tap into new income streams. This type of diversification is often associated with higher labor productivity, better economic prospects, and access to urban or peri-urban markets.

Loison (2015) categorizes livelihood diversification based on three dimensions:

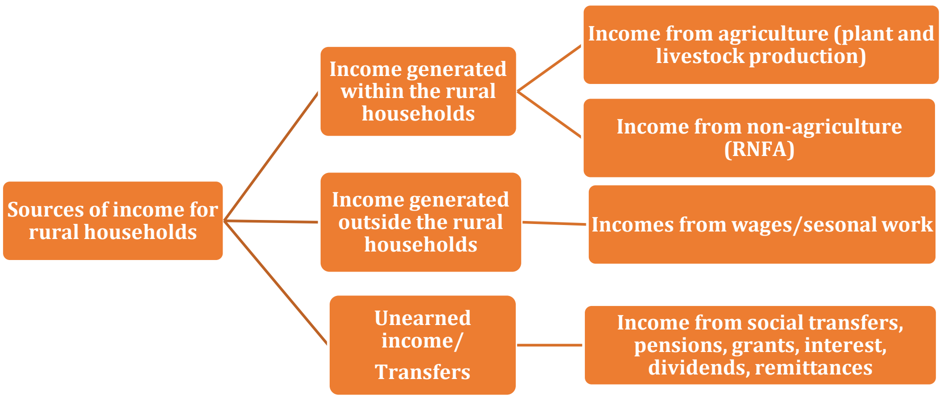
- Sector: Distinguishing between agricultural and non-agricultural activities.
- Function: Differentiating between wage employment and self-employment.
- Location: Considering whether activities are on-farm or off-farm.

This classification aids in large-scale analyses but may oversimplify the complexities of rural livelihoods, especially in regions where activities like non-timber forest product collection are prevalent.

Farm household income sources

According to the ILO's 2003 resolution (ILO, 2003), household income includes all regular monetary or in-kind inflows used for current consumption, excluding irregular or one-time sources that reduce net worth. Based on this, the research considers only income that: (1) is regularly recurring, (2) contributes to current economic well-being, and (3) does not arise from asset depletion or liabilities. Irregular payments such as lottery winnings, inheritances, and savings are excluded.

Rural household income can be categorized into three main sources. First, income within the household or farm includes primary agricultural production and diversified activities that utilize household resources. Second, income outside the household or farm stems from non-agricultural employment or work in external agricultural enterprises. Third, unearned income includes social transfers (such as pensions and grants), capital income (like interest and dividends), and remittances from family members (Pearce & Davis, 2001). This classification reflects the increasing complexity and diversification of rural livelihoods (Graph 1).



Graph 1. Framework of rural household income sources, adapted from Davis & Pearce (2001)

Characteristics of the surveyed regions

This study examines income diversification among rural households in North Macedonia, comparing the Polog and Pelagonia regions. These regions differ geographically, economically, ethnically and demographically:

Pelagonia Region, located in southern North Macedonia, is the country's largest but sparsely populated region (48.4 people/km^2 in 2018). It covers 18.9% of the territory and holds 11.1% of the national population, which is declining and aging. The average household has 3.3 members, and 86% of residents are ethnic Macedonians. In 2016, GDP per capita was slightly below the national average. Unemployment dropped from 19% in 2016 to 15.3% in 2018, with rural areas showing low rates due to outmigration. Agriculture is dominant, recognized by the production of cereals with the largest share of arable land (44%), tobacco, apples and milk production. Tourism potential lies in Prespa Lake, Pelister National Park and Krushevo. Despite its agricultural strength, income inequality is notable: in 2014, household income was 19% below the national average, and the gender pay gap reached 20% (SSO, 2019).

Polog Region lies in the northwestern part of North Macedonia and is the second most densely populated region. Unlike Pelagonia, it showed a slight population increase (0.5%) from 2016 to 2018. Households are larger (4.4 members), and Albanians form the majority (73%). The region has the lowest GDP per capita (46.2% of the national average) and high unemployment (29% in 2018). Still, net wages are relatively high, ranking second after Skopje. Agriculture is pasture-based (76% of total agricultural land), supporting livestock, alongside forage and vegetable cultivation. Polog has 21,216 farms, mostly small-scale. Its mountainous landscape and water resources offer significant potential for tourism and hydroenergy. In 2014, household income was 12.6% below the national average, with a narrower gender pay gap (9%) than in Pelagonia (SSO, 2019).

MATERIALS AND METHODS

This study utilized a quantitative comparative analysis approach to examine the socioeconomic and demographic characteristics, income structures and diversification patterns of rural households across two distinct regions in North Macedonia: Pelagonia and Polog. Data were collected from a stratified random sample of rural households, comprising a total of 140 households, 70 from each region, selected to represent the variability in household size, land holdings and economic activity. Data collection was conducted through direct, semi-structured interviews using a tailored questionnaire. In addition, two regional workshops were organized to discuss preliminary findings and validate the results with local stakeholders. To measure the level of income diversification among rural households, the Simpson diversity index (SDI) was applied. The SDI is a widely used measure that captures both the number of income sources and their relative distribution (Harishankar et al., 2022). A higher index value indicates greater diversification. Although several methods are available to assess income diversification, such as the Herfindahl Index, Shannon Index, and Entropy Index, the Simpson Index was selected due to its simplicity, intuitive interpretation, and frequent application in similar rural livelihood studies (Minot et al., 2006; Ibekwe et al., 2010).

$$SDI = 1 - \sum_{i=1}^{n} P_i^2$$

SDI=Simpson diversification index

n= total number of income sources and

Pi = Income proportion of the i-th income source

The Simpson diversity index (SDI) ranges from 0 to 1, where 1 indicates perfect diversification (income is equally distributed across all sources) and 0 indicates complete specialization (all income comes from one source). Based on Challa et al. (2019), the SDI ranges are classified as 0.00-0.38 for low diversification, 0.39-0.63 for medium diversification, and 0.64-1.00 for high diversification. To assess the normality and significance of the SDI, the Kolmogorov–Smirnov and Shapiro–Wilk tests were performed using SPSS software (Ghasemi & Zahediasl, 2012). Since the data did not meet the assumptions of normality, the non-parametric Mann–Whitney U test was applied (Nachar, 2008).

RESULTS AND DISCUSSION

Main characteristics of the rural households

The analysis of household characteristics across the Pelagonia and Polog regions, such as the official statistical data (Table 2), reveals distinct demographic and socioeconomic patterns. In terms of household composition, Polog demonstrates larger average household sizes of 5.0 members compared to Pelagonia's 4.1, with both regions showing relatively stable household structures as indicated by their similar coefficients of variation around 28%-29%. Examining the age distribution of household heads reveals significant regional variation. Polog's household heads are notably older, averaging 55.7 years compared to 48.0 years in Pelagonia, with Pelagonia showing greater variability in age distribution. This age disparity may reflect different migration trends, cultural norms regarding household leadership, or regional demographic transitions.

Agricultural land holdings present another area of regional contrast. Pelagonia households manage substantially larger land parcels, averaging 9.88 hectares reflecting a bigger area under extensive cereal cultivation, versus Polog's 6.91 hectares. Both regions exhibit high inequality in land distribution, with coefficients of variation exceeding 100%, indicating that a small proportion of households control disproportionately large land areas while many others work smaller plots.

Table 2. Household characteristics by regions

Category	Regions	Number of Rural Households	Mean	Max	Min	SD	CV
Household Size	Pelagonia	70.00	4.10	7.00	1.00	1.20	29%
	Polog	70.00	5.00	7.00	2.00	1.40	28%
	Total	140.00	4.60	7.00	1.00	1.40	30%
Age of Household Head	Pelagonia	70.00	48.00	76.00	22.00	11.20	23%
	Polog	70.00	55.70	77.00	31.00	9.90	18%
	Total	140.00	51.90	77.00	22.00	11.30	22%
Agricultural land ha	Pelagonia	70.00	9.88	47.00	-	10.12	103%
	Polog	70.00	6.91	32.00	-	7.73	112%
	Total	140.00	8.39	47.00	-	9.13	109%

Source: Authors' computation

The educational analysis (Table 3) shows that secondary education serves as the dominant qualification level across both regions, comprising 72% of all households. While

Pelagonia shows slightly higher rates of secondary (76%) and higher education (13%) compared to Polog's 69% and 11% respectively, Polog contains more households with only primary education (20% versus Pelagonia's 10%). The near absence of incomplete primary education (just 1% in Pelagonia) suggests basic education access is nearly universal, though higher education remains limited to about 12% of rural households overall.

Table 3. Education levels of rural household heads by regions

Education Level	Pelagonia	Share	Polog	Share	Total	Share
Incomplete primary edu.	1	1%	0	0%	1	1%
Primary education	7	10%	14	20%	21	15%
Secondary education	53	76%	48	69%	101	72%
Higher education	9	13%	8	11%	17	12%
Total	70	100%	70	100%	140	100%

Source: Authors' computation

The analysis of total household income sources (Table 4) reveals that agriculture is the primary contributor, with an average income of 14,809.75 EUR and moderate variability (CV=80%), indicating its central role despite some instability. Rural household activities generate significantly lower average income (3,121.9 EUR) but exhibit extreme variability (CV=230%), suggesting that only a few households benefit substantially (max=69,430.9 EUR), while most earn little or nothing from these sources. Wage income from outside the household and hired labor remains limited and highly unstable (an average of 1,304.9 EUR, CV=156%), reflecting weak labor market integration. Transfers and non-earned income also play a minor role in household budgets, with considerable disparities in access (average is 1,115.1 EUR, CV=122%). Despite the uneven distribution of individual income sources, total household income is relatively more stable, averaging 20,351.7 EUR with the lowest coefficient of variation among all categories. This suggests that households manage to balance different income streams to maintain a certain level of economic security.

In regard to the research region, this study examines regional disparities in household income sources between Pelagonia and Polog, revealing distinct economic patterns across key income categories. Polog demonstrates stronger agricultural performance, with a higher average income (18,461.9 EUR) and lower variability (CV=62%), indicating relatively stable farm productivity. In contrast, Pelagonia shows a lower mean agricultural income (11,157.6 EUR) and higher volatility (CV=98%), suggesting greater susceptibility to external shocks such as climate events or market fluctuations. Regarding rural non-farm activities, both regions exhibit extreme income disparities. Pelagonia records a higher mean income (4,837.1 EUR compared to 1,406.7 EUR in Polog), but also a much wider income range (0 EUR–69,430.9 EUR), pointing to unequal access to profitable ventures like handicrafts or small-scale production. The coefficient of variation further confirms this inequality, standing at 200% in Pelagonia and 152% in Polog.

External wage labor contributes minimally and remains unstable in both regions, with a coefficient of variation around 150%. Pelagonia reports slightly higher average earnings, 1,627.0 EUR vs. 982.8 EUR in Polog, yet neither region has a well-developed labor market outside the agricultural sector.

Transfer payments play a modest role in household budgets, averaging 1,234.3 EUR in Pelagonia and 996.0 in Polog. Variability is higher in Polog (CV=130%) compared to Pelagonia (CV=115%), reflecting inconsistent access to pensions, social transfers, or remittances.

When considering total household income, Polog exhibits greater overall stability, with a higher mean income (21,847.4 EUR) and lower variability (CV=47%). This reflects the region's more reliable agricultural base. On the other hand, Pelagonia faces higher economic vulnerability, with a mean income of 18,856.1 EUR and a higher coefficient of variation (CV=69%), due to instability in both farm and non-farm income sources.

Table 4. Analysis of household income sources, total and by regions: Pelagonia and Polog

Income sources in EUR	Regions	Mean	Max	Min	SD	CV
Income from agriculture	**Total**	14,809.7	49,951.2	-	11,788.3	80%
	Pelagonia	11,157.6	45,161.0	-	10,984.93	98%
	Polog	18,461.9	49,951.2	-	11,498.3	62%
Income from rural activities within the household	**Total**	3,121.9	69,430.9	-	7,189.2	230%
	Pelagonia	4,837.1	69,430.9	-	9,672.3	200%
	Polog	1,406.7	12,357.7	-	2,143.5	152%
Wage income outside the household and hired labor	**Total**	1,304.9	10,894.3	-	2,030.6	156%
	Pelagonia	1,627.0	10,894.3	-	2,415.9	148%
	Polog	982.8	4,878.0	-	1,503.1	153%
Transfers and non-earned income	**Total**	1,115.1	4,585.4	-	1,357.4	122%
	Pelagonia	1,234.3	4,585.4	-	1,419.4	115%
	Polog	996.0	4,195.1	-	1,291.5	130%
Total household income	**Total**	20,351.7	69,430.9	2,926.8	11,807.5	58%
	Pelagonia	18,856.1	69,430.9	2,926.8	13,056.9	69%
	Polog	21,847.4	53,122.0	6,325.2	10,287.3	47%

Source: Authors' computation

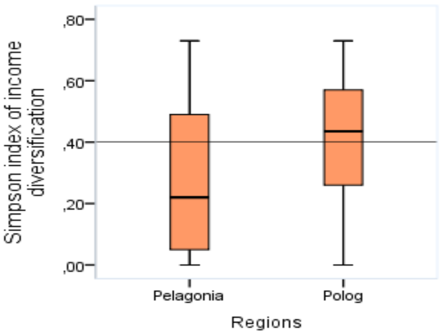
Analysis of the degree of rural diversification in the regions

The analysis of the Simpson Diversity Index (SDI) results (Table 5 and Graph 2) reveals distinct patterns of income diversification between the Pelagonia and Polog regions, with important implications for understanding rural livelihood strategies. Pelagonia demonstrates low income diversification with an average SDI of 0.27, falling within the 0.00-0.38 range classified as limited diversification. This indicates that most households in the region concentrate their income in just one or two primary sources, typically agricultural activities. In contrast, Polog shows medium-level diversification with an average SDI of 0.40, within the 0.39-0.63 range, reflecting a more balanced distribution of income across multiple sources that combines both agricultural and non-farm activities. Pelagonia exhibits greater variability in diversification strategies among households, as evidenced by its higher coefficient of variation of 85% compared to Polog's 50%. This wider dispersion indicates less consistency in income approaches across Pelagonia households.

Table 5. Simpson index of income diversification by region

Simposon index	Mean	Max	Min	SD	CV
Pelagonia	0.27	0.73	0.00	0.23	85%
Polog	0.40	0.73	0.00	0.20	50%
Total	0.34	0.73	0.00	0.22	65%

Source: Author's computation



Graph 2. Simpson Index comparison between Pelagonia and Polog Regions
Source: Author's computation

To determine whether a statistically significant difference existed in income diversification patterns measured by the Simpson index, between the surveyed regions, first the normality of the distribution for each region was evaluated. Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) were performed to assess the distribution of the index values (Ghasemi & Zahediasl, 2012). In Pelagonia region, both tests indicated a non-normal distribution (K-S: p=0.010; S-W: p=0.000). For Polog region, the Kolmogorov-Smirnov test suggested a normal distribution (p=0.073), but the Shapiro-Wilk test rejected normality (p=0.003). Given this inconsistency and the conservative nature of normality tests, the non-parametric Mann-Whitney U test was deemed appropriate for comparing the two regions (Nachar, 2008). This non-parametric test revealed a statistically significant difference (p=0.001) in income diversification between Pelagonia and Polog (Table 6).

Table 6. Results of normality tests and comparison of the Simpson diversity index between regions

Analysis	Pelagonia	Polog
Kolmogorov-Smirnov test	D=0.123, p=0.010*	**D=0.101, p=0.073**
Shapiro-Wilk test,	W=0.905, p=0.000*	**W=0.942, p=0.003***
Mann-Whitney U test	U=1635.5, Z=-3.402, p=0.001*	

Source: Authors' computation

These findings imply that households in Polog rely on a more varied mix of income sources, which may reflect differences in economic opportunities, agricultural constraints, or policy influences. Further investigation into the underlying drivers of this divergence, such as market access, infrastructure, or institutional support, could provide valuable insights for targeted rural development strategies.

CONCLUSIONS

This integrated analysis provides several fundamental insights about rural livelihood strategies in North Macedonia's distinct regional contexts. The research demonstrates that income diversification's effectiveness hinges not merely on the number of income sources but more critically on their inherent quality and stability. This principle manifests clearly in the contrasting regional patterns. Polog's medium level of diversification (SDI=0.40; CV=50%) represents an effective integration of complementary activities that together provide household stability, while Pelagonia's apparently lower diversification (SDI=0.27; CV=85%) conceals a problematic disintegration among unreliable income sources rather than representing true specialization.

The study highlights how household demographics and agricultural systems shape economic resilience in rural North Macedonia, specifically in the Polog and Pelagonia regions. In Polog, larger, multi-generational households with older heads have more stable and diversified income portfolios due to their accumulated experience and available labor resources. In contrast, Pelagonia's smaller households, led by younger individuals, struggle to establish sustainable diversification despite engaging in a broader range of activities. In the context of differing education levels between the two regions, shows that the higher education level and younger average age in Pelagonia correlate with a higher income from non-agricultural activities, presenting a promising potential that should be further developed. The agricultural systems also play a critical role: Polog's livestock-oriented production provides year-round income and supports more stable diversification, while Pelagonia's focus on seasonal cereal cultivation introduces vulnerabilities. These factors demand region-specific policy approaches. Polog would benefit from interventions that strengthen its existing diversification model through value chain enhancements, while Pelagonia needs efforts to stabilize its agricultural base and develop more reliable non-farm income sources. Both regions must address issues like land inequality and education-economy alignment, with tailored solutions suited to their specific conditions. The analysis also reveals that neither region achieves high diversification, indicating vulnerability to sector-specific shocks, such as agricultural volatility in Pelagonia or reliance on primary income sources in Polog. Thus, the study emphasizes the need for rural development strategies that consider the unique demographic, agricultural and economic characteristics of each region.

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