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Investigation of the local pear genotypes grown in Malatya

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Abstract
This study was carried out between 2020 and 2021 on local pear genotypes grown in the Malatya region (Türkiye), especially in the yard of Apple-Pear Germplasm of Malatya Turgut Özal University. In the study, phenological, pomological, and phytochemical characteristics of nine pear genotypes were analyzed. The following dates were identified for the selected characteristics: the bud swell between March 9 and 27, the bud bursts between March 14 and April 1, the start of flowering between March 28 and April 7, full bloom between April 2 and 11, the end of flowering between April 6 and 15, harvest between August 10 and November 13, defoliation between December 3 and 17, and the number of days from full bloom to harvest ranged between 128 and 216 days. The following results were obtained: fruit weights were 46.3-202.6 g, fruit lengths were 44.8-91.2 mm, fruit diameters were 43.8-76.2 mm, fruit flesh firmness were 2.4-9.62 kg/cm^2, fruit volume was 45.5-204.3 ml, and fruit color values were 21.3-77.1 as *L* value, -2.49-0.1 *as a* value, and -2.03-54 *as b* value. The water-soluble dry matter content of the genotypes ranged between 10.3-15.9, pH values ranged between 3.99-5.37, total phenolic content ranged between 167-992 mg GAE /1000g, and total antioxidant activities ranged from 284 to 1454 mg TEAC/1000 g. Aliseydi from the summer pear genotypes and Parlak and Armut1 genotypes from the autumn genotypes were important. It is thought that Biber, Dudunun and Efendi genotypes from winter genotypes may be important materials for future studies.
Keywords: *Pyrus comminus*, Local pear, Phenology, Pomology, Antioxidants

INTRODUCTION

As a member of the pome fruit group, the pear belongs to the genus *Pyrus* L. the subfamily *Pomoideae* of the family Rosaceae of the order Rosales. This genus contains many distinct species. Considering pomiculture, 13 species have gained significance in developing as fruit cultivars and using rootstocks (Özbek, 1978). *P. communis* L. is one of the most significant species of pears and has spread over a broad geographical area. It also plays a critical role in developing pear cultivars (Özbek, 1978). The number of local pear varieties in Türkiye, one of the gene centers of *P. communis*, reportedly exceeds 600 (Özbek, 1947; Davis, 1972).

Ülkümen (1938) states that Türkiye constitutes a significant fruit production region. He also emphasizes that pears and apples are grown in Türkiye, especially in eastern and western provinces.

According to the most recent statistics, the global pear production is 39.210.669 tons annually in an area of 135.190.430 decares. Türkiye also ranks fifth among pear-producing countries with a production figure of 545.569 tons which corresponds to 1.4% of the total in an area of 260.707 decares. Türkiye's pear

production is growing in tandem with global pear production (FAO, 2022).

The province of Malatya is known for apricot production and has considerable potential for producing other fruit species that grow best in temperate climates. According to 2021 production statistics in Malatya province, the pear yield was 40 kg per tree, amounting to a total production of 6.635 tons (TUIK, 2021). In Battalgazi district, where the current study was conducted, the pear yield in 62 decares was 356 tons (TUIK, 2021). There are local varieties in addition to the well-known standard pear cultivars.

In Türkiye, which has a diverse fruit-growing culture, there are many explicit local varieties in pear production, along with the standard cultivars, which are economically worthless in many regions and generally appraise at domestic consumption or in local markets. Besides, these species create a different taste and serve as an exceptional source of genetic material for breeding studies, constituting a significant genetic source to develop new varieties. The larger the fruit population, the more likely it is to find the desired material in breeding studies. Preserving local cultivars and wild species can ensure the continuity of crop production, while it is also important to protect wild species and local genotypes to achieve sustainable crop production. In this context, there are numerous studies in Türkiye on protecting genetic resources. The pomological studies have also revealed the value of fruit varieties (Doğan and Güleryüz 2001; Bayındır 2017).

The aim of this study is to reveal the local pear varieties grown in Malatya province and its districts, which have an important place in terms of cultural fruit growing and natural resources, to bring them into the economy, to preserve many pear genotypes, which are about to disappear and have genetic and commercial value, and to use them as a genetic material.

MATERIALS AND METHODS

Materials

This study was conducted between 2020 and 2021 in the yard of Apple-Pear Germplasm of Malatya Turgut Özal University, Faculty of Agriculture, where the local pear genotypes which are well-known to the local population and have been grown for many years have been planted. The genotypes in the Germplasm parcel were created with superior genotypes determined from the genotypes grown in the province of Malatya and districts within the scope of the Project of Identification and Conservation of Malatya's Important Genetic Resources (Asma et al.,2003). In 2020 and 2021, bud swell, bud burst, beginning of flowering, full flowering, end of flowering, eating maturity were observed from tress belonging to 9 genotypes (Abbas, Aliseydi, Armut1, Biber, Dududnun, Efendi, Hacı Hasan, Kış and Parlak pear) determined by preliminary selection. Harvest and leaf fall dates were determined. Fruit weight, fruit length, fruit diameter, fruit flesh firmness, fruit volume, fruit color values, amount of water-soluble dry matter, Ph, total phenolic content, and total antioxidant activity were assessed in 30 fruits randomly taken from the determined trees in analysis of both years.

Methods

Both the pomological analyses and phenological observations took place in 2020. However, the phenological and pomological characteristics of the fruits collected in 2020 from the local pear genotypes were analyzed in 2021.

The pH was measured using a pH meter and the amount of Water-Soluble Dry Matter was observed using a hand refractometer.

The Folin-Ciocalteu method was used to determine total phenolic content and the results were expressed as gallic acid equivalent. 50 µL of extract, 950 µL of distilled water, and 1 mL of Folin-Ciocalteu solution were added into a tube and waited for 3 minutes. Then, 1 mL of 2% Na2CO3 solution was added and waited for another 1 hour in the dark. The absorbance of the solution was measured at a wavelength of 765 nm and the results were expressed as gallic acid equivalent.

ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging assay was applied to determine antioxidant capacity. ABTS of 0.0384 g was weighed and dissolved in 2 mL of distilled water. After adding 2 mL of potassium persulfate solution, it was completed up to 10 ml with distilled water. After keeping the solution in the refrigerator for 1 night, its absorbance was adjusted with methanol to give an absorbance of 0.700 at 734 nm. 0.40 mL of the extract and 2.96 mL of the adjusted ABTS solution were added into a tube and the absorbance was measured at 734 nm. Results are expressed as trolox equivalents.

SPSS 25.0 statistical software was used to interpret the data. In order to determine the differences between the tested applications, ANOVA or DUNCAN test was applied at the significance level of 0.05.

RESULTS AND DISCUSSION

The observation-based findings of the nine genotypes between 2020 and 2021 revealed that in both years, bud swell initially began between March 9 and 13 (Aliseydi) and ended between March 20 and 27 (Kış). The earliest bud burst occurred between March 14 and 19 (Aliseydi) and the latest bud burst occurred between March 31 and April 1 (Kış). While the first flower bloomed between March 28 and April 1 (Aliseydi), the last flower bloomed between March 31 and 7 April (Kış). Additionally, the initial full bloom was observed between April 2-5 (Aliseydi) and the latest full bloom took place between April 6 and 11 (Kış). Considering the end of flowering dates between 2020 and 2021, dates of April 6–9 (Aliseydi) and April 11–15 (Kış) were the first and last observed days for the end of flowering, respectively. The genotypes that reached the earliest harvest maturity in 2020 were Aliseydi and Hacı Hasan (August 10), whereas their harvest dates for 2021 were August 15-22. On the other hand, the genotypes that reached the last harvest maturity in 2020-2021 were Kış and Armut1 (between October 22 and November 13). The shortest period between full bloom and harvest identified among the genotypes in both years was 128-137 days (Hacı Hasan), and the longest period was 202-216 days (Armut1) (Table 1, 2).

According to the observations, in both years bud swell occurred at the earliest (March 9-13) in the Aliseydi pear genotype and at the latest (March 20-27) in the Kış pear genotype, bud burst occurred at the earliest between March 14 and 19 (Aliseydi) and at the latest between March 31 and April 1 (Kış). There was a difference of 4-11 days between the dates of bud swell and bud burst in both years. The average temperature of March 2021, when bud swell and bud burst were observed, decreased by 2.5 °C compared to March 2020 and the differences were observed in these phenological stages.

Table 1. Phenological observation dates for 2020 and 2021.

Genotypes	Bud Swelling		Bud Burst		First Flower Bloom		Full Bloom	
	2020	2021	2020	2021	2020	2021	2020	2021
Abbas	13.03	19.03	20.03	30.03	30.03	05.04	03.04	08.04
Aliseydi	09.03	13.03	14.03	19.03	28.03	01.04	02.04	05.04
Armut1	13.03	21.03	20.03	31.03	29.03	07.04	03.04	11.04
Biber	13.03	23.03	20.03	31.03	30.03	06.04	03.04	11.04
Dudunun	15.03	25.03	20.03	31.03	30.03	07.04	03.04	11.04
Efendi	13.03	23.03	20.03	31.03	30.03	05.04	03.04	08.04
Hacı Hasan	13.03	20.03	20.03	26.03	31.03	04.04	04.04	07.04
Kış	20.03	27.03	25.03	01.04	31.03	07.04	06.04	11.04
Parlak	10.03	25.03	20.03	31.03	29.03	06.04	04.04	11.04

Table 2. Phenological observation dates for 2020 and 2021.

Genotypes	End of Flowering		Harvest		Leaf Fall		S.P	
	2020	2021	2020	2021	2020	2021	2020	2021
Abbas	07.04	12.04	03.10	10.10	7.12	11.12	183	185
Aliseydi	06.04	09.04	10.08	15.08	03.12	10.12	131	136
Armut1	07.04	14.04	22.10	13.11	08.12	15.12	202	216
Biber	07.04	14.04	-	10.10	07.12	15.12	-	186
Dudunun	07.04	14.04	25.09	29.09	07.12	15.12	172	171
Efendi	07.04	11.04	-	10.10	14.12	08.12	-	185
Hacı Hasan	08.04	12.04	10.08	22.08	07.12	11.12	128	137
Kış	11.04	15.04	22.10	13.11	10.12	17.12	201	216
Parlak	07.04	12.04	03.10	10.10	7.12	11.12	183	185

S.P: The shortest period between full bloom and harvest

The measurements made in 2020 and 2021 revealed that the highest fruit weight of the pear genotypes ranged from 202.59 (Armut1) to 175.79 (Parlak), respectively. However, the lowest fruit weight in both years ranged between 56.96 and 46.35 (Hacı Hasan). The fruit length of pear genotypes ranged from 44.78 mm (Kış) to 91.18 mm (Parlak), and the average fruit diameter ranged between 43.78 mm and 76.17 mm. Fruit flesh firmness ranged from 2.44 kg/cm^2 (Aliseydi) to 9.62 kg/cm^2 (Abbas) and the fruit volume ranged between 45.48 and 204.34 ml. Considering the lowest and highest values, the L* color values were 21.29-22.24 (Kış) and 76.93-77.63 (Aliseydi), and the value on the green-red scale was -2.49 -1.92 (Hacı Hasan) and -0.2 (Dudunun) in 2020 and -1.92 (Hacı Hasan) and 0.1 (Kış) in 2021. Similarly, the b value on the blue-yellow scale varied between 1.32 (Kış) and 53.64 (Aliseydi) in 2020 and -1.97 (Abbas) and 53.96 (Hacı Hasan) in 2021. Among the genotypes analyzed, the lowest and highest water-soluble dry matter contents were

10.3-10.8 (Dudunun and Hacı Hasan) and 14.6-14.3 (Aliseydi), respectively. However, the lowest and highest pH values were 3.95-3.99 (Parlak) and 5.37-5.31 (Aliseydi), respectively. While the lowest total phenolic content was 168-221 mg/1000 g GAE (Aliseydi-Dudunun), the highest total phenolic content was 814 -849 mg/1000 g GAE (Kış-Efendi). The antioxidant activity analysis results of pear genotypes also revealed that it ranged between 208 mg/1000 g TEAC and 1454 mg/1000 g TEAC in 2020; whereas, it remained between 275 mg/1000 g TEAC and 1201 mg/1000 g TEAC in 2021 (Tables 3, 4, and 5, Figures 1, 2, 3, and 4).

Table 3. Pomological Characteristics of Pear Genotypes in 2020 and 2021

Genotypes	Fruit Weight (g)	Fruit Length (mm)	Fruit Diameter (mm)	Fruit Flesh Hardness (kg/cm²)	Fruit Volume (ml)
Abbas	101,03±20,39b	60,87±4,57c	60,26±4,08c	8,34±1,59a	126,14±6,42b
Aliseydi	101,77±16,55b	53,18±3,29d	61,7±2,36c	2,41±0,36e	109±7,71c
Armut1	202,59±40,17a	62,46±5,37c	76,17±13,71a	7,63±0,63b	135,27±7,29b
Biber	0	0	0	0	0
Dudunun	101,34±20,68b	68,13±7,59b	59,64±4,66c	5,35±0,67d	106,19±2,09c
Efendi	0	0	0	0	0
Hacı Hasan	56,96±4,07c	48,76±2,17e	44,49±4,02e	2,97±0,69e	54,09±5,93e
Kış	66,84±17,68c	46,89±4,95e	50,23±4,45d	8,14±1,26a	92±0d
Parlak	183,44±53,54a	91,18±6,99a	70,17±4,72b	6,56±0,83c	200,17±32,82a

Table 4. Pomological Characteristics of Pear Genotypes in 2020 and 2021

Genotypes	Fruit Weight (g)	Fruit Length (mm)	Fruit Diameter (mm)	Fruit Flesh Hardness kg/cm²)	Fruit Volume (ml)
Abbas	131,49±35,2b	64,3±4,2c	68,66±5,08ab	9,62±1,62a	129,34±5,17b
Aliseydi	72,25±16,15cd	46,56±5,92e	51,62±4,77d	2,44±0,37c	83,27±13,11d
Armut1	139,01±30,3b	59,48±4,98d	71,06±7,91a	9,13±1,57a	131,4±6,02b
Biber	133,14±48,19b	55,29±7,69d	62,81±7,83c	6,53±0,7b	116,67±4,93c
Dudunun	83,55±33,06c	57,3±5,95d	51,88±5,94d	5,73±1,05c	84±0d
Efendi	156,17±41,87ab	74,64±7,41b	66±6,49cb	7,05±0,52b	126,74±6,68b
Hacı Hasan	46,35±8,88d	47,67±4e	43,78±3,67e	2,93±0,58c	45,48±4,06e
Kış	67,84±15,95cd	44,78±3,92e	50,21±2,95d	7,82±0,82a	66,66±0e
Parlak	175,79±60,64a	80,73±7,6a	70,21±5,55ab	6,06±1bc	204,34±16,55a

Table 5. Pomological Characteristics of Pear Genotypes in 2020 and 2021

	Color					
	2020			2021		
Genotypes	L	a	b	L	a	b
Abbas	21,51±0,83b	-0,2±0,03a	-1,2±0,05c	22,1±0,71b	0,05±0,02a	-1,97±0,04b
Aliseydi	76,93±3,01a	-1,6±2,54b	53,64±3,79a	77,63±3,45a	-1,92±1,25b	51,66±8,81a
Armut1	21,79±1,04b	-0,12±0,16a	-1,22±0,08c	22,05±0,79b	-0,06±0,17a	-2,03±2,71b
Biber	0	0	0	21,99±0,41b	0,04±0,04a	-1,92±0,04b
Dudunun	21,33±0,79b	-0,2±0,02a	-1,21±0,08c	21,76±0,76b	-0,09±0,06a	-1,47±1,2b
Efendi	0	0	0	21,59±1b	0,12±0,13a	-1,95±0,1b
Hacı Hasan	73,95±14,77a	-2,49±0,59b	53,2±3,49a	77,1±3,12a	-1,3±2,85a	53,96±3,4a
Kış	21,29±0,54b	-0,23±0,03a	-1,32±0,3c	22,24±0,4b	0,1±0,05a	-1,96±0,06b
Parlak	25,75±2,08a	-1,27±0,25b	-04,03±0,29b	21,71±0,92b	-0,03±0,02a	-1,96±0,12b

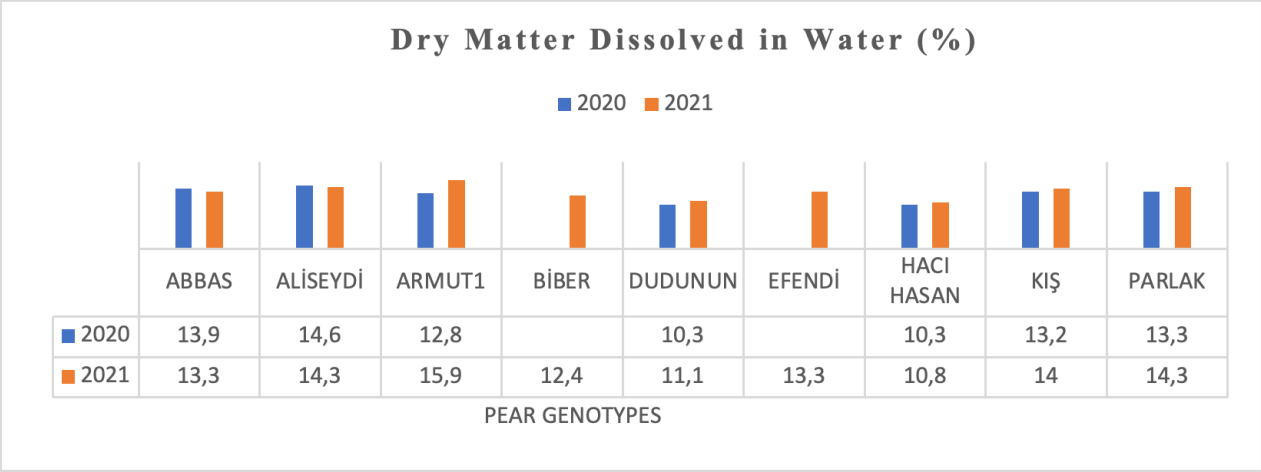

Figure 1. Dry Matter Dissolved in Water for values 2020 and 2021

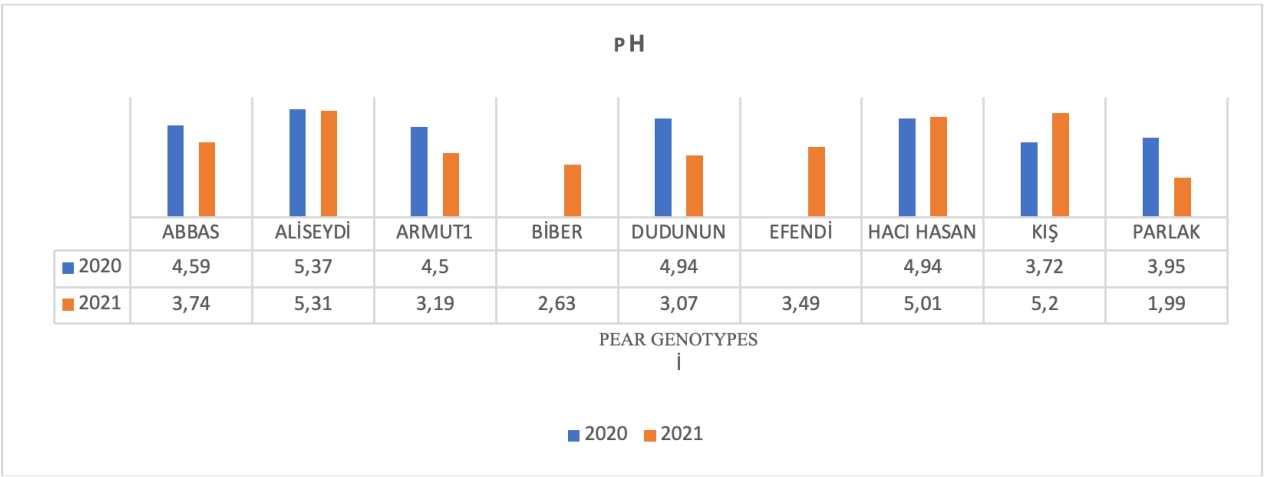

Figure 2. pH for values 2020 and 2021

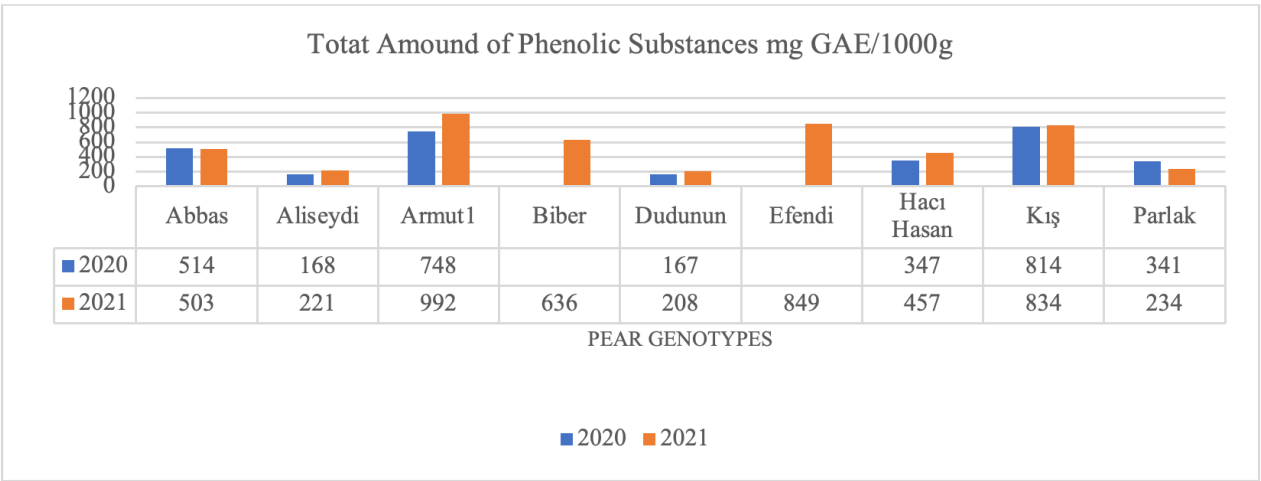

Figure 3. Totat Amound of Phenolic Substances for values 2020 and 2021

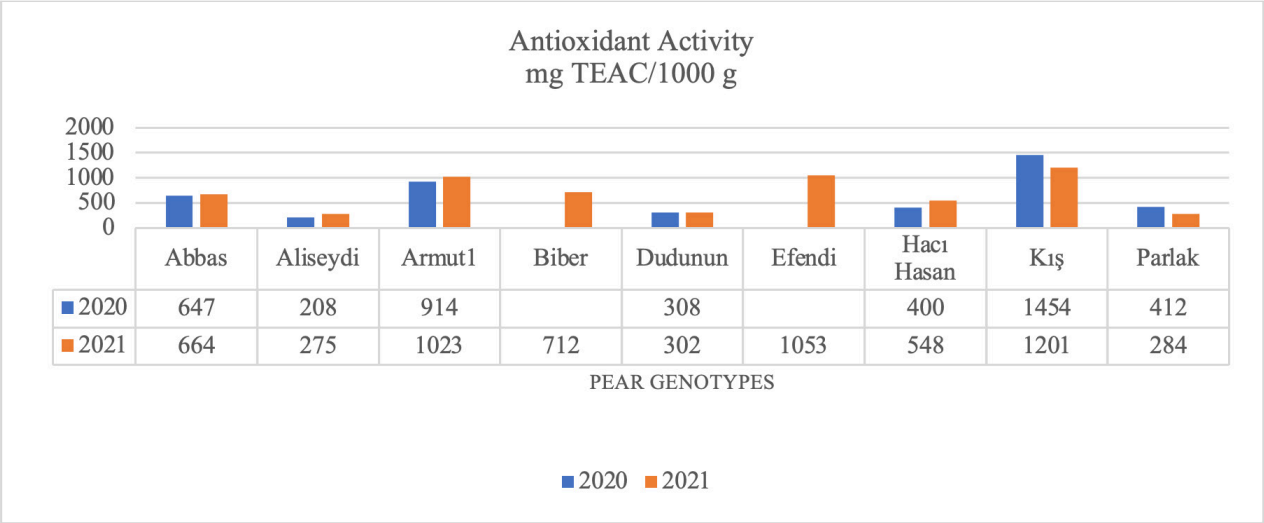

Figure 4. Antioxidant activity values for 2020-2021

Considering the previous studies conducted in Türkiye, the weight of pear species grown in Ünye district of Ordu province in 2012 was between 18.7 g and 258.3 g (Bostan and Acar, 2012) in Gürgentepe district of Ordu province, and the phenological, morphological and pomological characteristics of the local pear species grown in these districts were observed. A study conducted to identify some fruit characteristics of early pear (Pyrus communis L.) types grown in the Eğirdir district reported that the fruit weight ranged between 36.23 g and 146.65 g (Kılıç, 2015). Polat and Bağbozan (2017) reported the average measurements for fruit weight as 21.6-273 g, fruit width as 35.8-73.5 mm, and fruit length as 25.9-117.3 mm. Additionally, the water-soluble dry matter content of the fruits was 10.6-16.3%, TEA content was 0.1-0.9%, and fruit pH ranged between 3.2 and 5.4. As a result, they found that Yellow Pear and E2470 were promising among all pear genotypes. In another study focusing on selecting local pears grown in Malatya province, Bayındır et al., (2019) identified three promising genotypes based on the pomological analysis of the fruits. They accordingly reported that the fruit weight, fruit flesh firmness, water-soluble dry matter content, titratable acidity, and the pH measurements of these genotypes were between 121.8-163.98, 4.18-8.35 kg/cm^2, 13.60%-15.40%, 0.18%-0.21%, and 3.95-4.83, respectively. Mete and Seferoğlu (2019) observed that full bloom occurred between March 9 and 25, the number of days from full bloom to harvest varied between 101 days (Santa Maria) and 202 days (Ankara). In a study conducted by Murathan et al., in 2019, the total phenolic content of şakok pear was determined as 174.2 mg GAE/100g, and the ABTS radical scavenging activity was 48.2%. Furthermore, Turalı and Karadeniz (2020) reported bud swell between March 07 and April 5, the end of flowering between April 13 and May 9, and harvest between August 21 and October 20. They additionally found that the water-soluble dry matter content ranged between 7.50%-16.50% and 8.50%-15.50% and the pH value of the pears ranged between 2.82 and 6.12.

It was observed in the present study that the data obtained from the genotypes were compatible with other studies. One of the most important parts in pomology is fruit weight. While Armut1 (202.59g) and Parlak (175.79g) pear genotypes ranked first in terms of fruit weight, Hacı Hasan pear genotype, which was recorded as a summer genotype, had the lowest fruit weight in both years. When evaluated from a pomological perspective, two genotypes stand out (Parlak, Armut1). It was determined that the Parlak pear genotype, which is one of the genotypes with remarkable pomological characteristics, had a medium firmness and the fruit variety of the Armut1 genotype was higher than that of the Parlak pear genotype. The genotype with the highest fruit variety was the Abbas pear genotype. In terms of storage conditions, it remained intact for 2 months at 4°C.

CONCLUSION

Ensuring sustainable plant production can be possible only by preserving wild species. The current study conducted with nine genotypes with no commercial value is thought to set a precedent to prevent the extinction of local pear cultivars, identify their breeding characteristics, and select the superior ones for cultivation purposes. The current study, on the other hand, revealed that Aliseydi a summer genotype and Parlak and Armut1 the autumn genotypes are promising cultivars, additionally highlighting that the autumn genotypes, such as Biber, Dudunun, and Efendi, may also serve as considerable breeding materials for future studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

This article is derived from Hazal KARACA's Master thesis. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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RESEARCH ARTICLE

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The contributions of smallholder farmers toward household food security in Chabelane village, Limpopo province, South Africa

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Abstract

Although South Africa is considered to be food secure at the country level, large numbers of households within the country remain food insecure (De Cock et al. 2013). Food inaccessibility in many rural areas of South Africa has manifested itself in many formats but has positioned poor households to struggle to meet their basic household requirements and be more vulnerable to food insecurity. Details of such food insecurity, however, may differ (De Cock et al. 2013; D'Haese et al. 2013). This study aimed to investigate the contributions of smallholder farmers toward household food security in Chabelane Village, in Limpopo Province, South Africa. A quota sampling method was used to select 20 smallholder farmers in Chabelane Village. Data was collected through semi-structured interviews and the analysis of data was done using thematic analysis. This study has highlighted the prevalence of food security status among smallholder farmers. Household food security in rural areas is a significant matter, as it is necessary to have appropriate access to healthy foods that lead to active life. Furthermore, the study highlighted the smallholder farmers' agricultural practices which are crop farming and livestock farming. Moreover, increased food supply, job opportunities, and income generation were highlighted as the contributions of the smallholder farmers toward household food security. However, the smallholder farmers in this study area experienced challenges such as lack of finances, water shortages, and lack of access to formal market. This study recommends access to financial aid through access to loans from the banks, raising awareness among smallholder farmers about the knowledge on how to access formal markets. Additionally, the Department of Agriculture or local municipality to provide water tanks or boreholes for smallholder farmers to have undisrupted access to water for productive farming practices. This study contributes to the growing body of knowledge on the contributions of smallholder farmers towards household food security by providing valuable insights into the relationship between food security and smallholder farmers, particularly in Chabelane Village, Limpopo Province, South Africa.

Keywords: Food security, Smallholder farmers, Chabelane Village, Households, South Africa

INTRODUCTION

FAO, IFAD, UNICEF, WFP, and WHO (2022) estimate that 828 million people worldwide were hungry in 2021, with Africa (278 million) and Asia (425 million) suffering the greatest losses. Due to the location of the bulk of the world's poor countries, the continent of Africa faces greater issues related to food insecurity than any other. Food security issues impede the development of most impoverished nations. Sub-Saharan Africa is one of the regions that face issues

related to food security because the majority of the world's food insecure population resides in this region. Compared to earlier, food security is now given more attention in the policy program. This significance can be attributed to the extent, scope, and quantifiable proof of food insecurity. The nature of food insecurity's irregularity is a serious worry on a global scale, but it is not region- or country-specific (Nkegbe et al., 2017).

Furthermore, the global issue of food security continues to be of concern (Food and Agriculture Organisation, 2014). According to the FAO's "State of Food Insecurity and Nutrition in the World 2023" study, 600 million people will be chronically undernourished by 2030, while up to 783 million people experienced hunger in 2022. There are undernourished people in both industrialized and developing parts of the world, but the distinction between the two comes down to the size and intensity of the issue as well as the percentage of the population that is impacted.

In addition to guaranteeing the quantity of food, achieving food security entails assuring food quality and continuity for every member of the household (World Bank, 2010). Any nation's ability to thrive over the long run can be positively impacted by having enough nutrition and food security (Holben, 2010). According to Turyahabwe et al. (2013), home poverty and food insecurity result from a failure to meet a child's daily nutritional needs as well as worry about one's future ability to generate or obtain food. A common understanding is that improving household food security is critical to raising the standard of living for the impoverished in rural areas.

The definition of food security also makes it abundantly evident that, despite the nation's ability to produce or import enough high-quality food annually to satisfy the necessary demand for both public and private distribution, the concept of the food problem is a significant one with numerous facets (Shahjhan et al. 2016). This could not be the case for food security in Greater Letaba Local Municipality households, though, as there are few studies on the topic particularly on the contributions of smallholder farmers towards household food security. The study, therefore focused on the contributions of smallholder farmers toward household food security in Chabelane Village, Limpopo Province. This study also focused on the prevalence of food security status among smallholder farmers' households and the challenges smallholder farmers encounter which pose a threat to households' food security in Chabelane Village.

Research Objectives

The main objective of this study was to investigate the contributions of smallholder farmers toward household food security in Chabelane Village, Limpopo Province. The study also aimed at the following specific objectives:

1. To assess the prevalence of food security status among the smallholder farmers' households in Chabelane Village.

2. To determine the extent to which smallholder farmers contribute to household food security in Chabelane Village.

3. To investigate challenges faced by smallholder farmers toward achieving household food security in Chabelane Village.

Research Questions

Main research question

The main research question on which the study is based on is as follows: what are the contributions of smallholder farmers toward household food security in Chabelane Village, Limpopo Province?

MATERIALS AND METHODS

Study Area

The study was conducted in Chabelane Village, in Limpopo Province, South Africa. Chabelane Village lies within 23 ° 42 ′ S, 30 ° 8 ′ E in the Sekgosese area, Mopani District under Greater Letaba Municipality (GLM). GLM is a Limpopo Province Category B municipality. It is the smallest municipality in the district with a 1,891 km2 area and rainfall of 300-600 mm annually (GLM IDP 2021/2026). The municipal territory consists of 132 rural communities of which Chabelane Village is one of them (GLM IDP 2021/2026). The Limpopo Province is known for its plentiful agricultural area and is among the primary agricultural production regions in the country, recognized for its livestock, fruits and vegetables, grains, and tea (Stats SA, 2019). According to the Department of Agriculture and Rural Development (2016), agriculture is one of the Limpopo's major economic pillars.

Research Methodology

This study employed a qualitative research technique. Qualitative research entails a variety of interpretative tools to deduce, describe, and translate certain occurrences that occur in the natural world, and therefore can be summarized as a type of descriptive research (Creswell, 2009). The qualitative research approach was used in this study because 'it situates the researcher in the real world and incorporates interpretive behaviors that make the world visible' (Flick,

2014:10). The qualitative method enabled the researcher to investigate the issue in question and develop a thorough understanding of the study's basic issue (Creswell, 2013).

Sampling and sampling procedure

This study employed a non-probability, quota random sampling to select the participants. Quota random sampling entails grouping the population of the research into segments (Agbenyegah, 2013). The usage of quota sampling ensures that the sample group contains specific traits of the population the researcher has chosen (Dudovskiy, 2016). The study comprised of 20 smallholder farmers who are directly practicing smallholder agriculture activities in the Chabelane Village.

Data Collection Tool

This study employed semi-structured interviews to collect data from 20 smallholder farmers in Chabelane Village for a period of three months. The interviews took place from August 2022 to October 2022. All interviews were recorded digitally and transcribed.

Data Analysis Method

Thematic analysis was employed in this study for data analysis. All steps addressed by Braun and Clarke (2006) were applied in this study's data analysis. According to Caulfield (2019), thematic analysis was used to discover common themes in a collection of texts, such as interview transcripts. Thematic analysis entails reading and evaluating all material obtained to code, organize, and link diverse concepts (O'Connor & Gibson, 2003). The transcribed interviews were carefully reviewed, and key themes related to the contributions of smallholder farmers toward household food security were identified. The identified themes were then organized into a table to provide a comprehensive overview of the smallholder farmers' contributions, the challenges, and the prevalence of food security status as themes in a column and another column labeled "sub-themes".

Ethical considerations

The study complied with ethical guidelines by ensuring that every participant gave their consent before participating in the study, and the researcher ensured participants' confidentiality and anonymity. Ethical approval for this study was received from the University of Pretoria and the Faculty of Humanities Research Ethics Committee, numbered (HUM008/0722). All ethical rules and standards by the research institution were adhered to.

RESULTS AND DISCUSSION

According to the qualitative analysis method, three main themes emerged from the data collected. The first-order themes (specific deduction) are grouped under three headings. These are the prevalence of food security in Chabelane Village, the contributions of smallholder farmers toward household food security in Chabelane Village, and the challenges smallholder farmers experience. Each specific deduction (response) is also a summary of the most repetitive general deductions in smallholder farmer interviews and is detailed in almost all of the research areas (Table 1). These findings furnished invaluable insights into the contributions of smallholders towards household food security in Chabelane Village, Limpopo Province, South Africa.

Smallholder farmers' agricultural practices:

Types of farming practiced

The data in this study indicated that 24% of the participants practice livestock farming, 37% of the smallholder farmers practice crop farming, and 30% practice both crop farming and livestock farming. The findings illustrated that the majority of smallholder farmers are still practicing mostly crop farming rather than livestock farming. In crop production, households are engaged in additional strategies for enhancing food security and food availability in their households. The crops grown by smallholder farmers are intended for domestic consumption, and the household can become either partially or completely food insecure (Mutisya et al. 2016). Rose (2008) asserted that the development of staple food crops has a role in ensuring the food security of households. Vegetables, maize, and beans are only a few of the many crops that may be grown in South Africa (Gbetibouo & Hassan (2005). For this reason, the majority of the country's soil is appropriate for producing these kinds of crops. On the other hand, livestock is seen as an aspect of security and a means of coping during crop failure and other disasters (Goulet, 2013). Since livestock is a commodity and can be used as a reserve that can be converted into money when required, almost all rural households in nations that are developing own some livestock (Ali & Khan, 2013). According to Abu and Soom, (2016), several researches have shown that households that own cattle are more likely to engage in ecologically friendly farming practices and have sufficient food security. Diao et al. (2021) found that an increase in livestock increases a household's probability of being nutritious by about 51% in rural Punjab, Pakistan.

*I am a crop farmer, am not into livestock farming because I do not have any livestock at the moment. I plant vegetables which include cabbage, onions, chilies, maize, sweet potatoes, beetroot, lettuce, baby marrow, and pumpkin. I plant vegetables because I want to eat healthy and fresh food (*Participant 19).

I practice crop farming only in my yard; not so long I will be venturing into livestock soon. I plant maize crops, ground nuts, beans, and green leafy vegetables (Participant 12).

*Most of the time on my farm I plant spinach, tomatoes, and maize and I have also an orange tree. I only do crop farming, other farming ideas I have not yet thought about them(*Participant 20).

Farming area and size

Land availability is a significant aspect in influencing smallholder agriculture's success. The data revealed that 63% of the participants practice farming 200 meters away from their homes, and 28% indicated they farm in their yards. These results are consistent with those of Mashamaite (2014), who discovered that most participants utilized portions of their less than 500 square meter home stands for farming. The size of the farm could have a negative or positive impact on the level of productivity. This supports the argument by Hendricks (2014) that most of the land redistribution has been unproductive and non-functional and that subsistence farmers still produce a quarter of what commercial farmers produce. This confirms the arguments that subsistence agriculture's production involves mainly households producing on relatively small plots of land less than one hectare with limitedresources for household subsistence. According to Baiphethi et al. (2009), smallholder farmers have unsustainable tiny and diminishing farm sizes as well as poor-quality land, this is a result of problems with ownership of land which have to be fixed. In most cases, smallholder farmers run their fields under ambiguous customary land ownership, because of their erratic land tenure systems, they are unable to make the most use of the land. Insecure property rights, for example, do not encourage farmers to invest in land, which would increase production.

My farming takes place outside my yard, which is located 1 kilometer from where I stay, the farm size is 1 hectare (Participant 20).

My yard is enough for me to stay in and to plant. I have about 100 meters square, which I use for crop farming (Participant 17).

I practice farming here in my yard, my stand size is quite big, half of it I use for crop farming, and I also erected two kraals for my cattle and goats (Participant 19).

I use my yard to cultivate crops and other space I erected two kraals for the livestock. The farming area is approximately 500 hectares. I started this because I was bored but now, I see is helping me to get a few tomatoes (Participant 12).

Household Food prevalence status:

According to StatsSA (2020), South Africa currently has one of the greatest wealth inequalities in the world because of the persistent financial gaps across sub-population groups, the nation has static household food insecurity levels and nutritional difficulties among sub-population groups like women, children, and rural households, the majority of whom are from low-income families (StatsSA, 2020). According to Du Toit (2014), the problem with food access in South Africa is real, most people don't have enough access to nutritious meals at home. Food insecurity among South African households appears to have increased (Van Den Berg & Raubenheimer, 2015). In this study, the Household Dietary Diversity Score was used to classify households either into food secure or insecure categories.

Food consumed per day for the past week (7 days)

The data indicated that 36% of the participants reported that they had more than 5 types of food under the 12-food group that contains a variety of nutritional dietary food. These participants stated that they consumed 3 meals a day (breakfast, lunch, and supper) while 73% mentioned that they ate 2 meals a day (breakfast and supper while others ate lunch and supper). They further indicated that they had less than 5 types of food that they had consumed in the previous 7 days. In a study conducted by Machete (2020), it was found that nearly 3 in every 10 participants reported having 2 meals a day (lunch and supper only).

During the past week, I ate 3 meals in a day, I ate breakfast, bread, milk, eggs, tea, and butter, and the other day it was porridge. Then around 13: 00, I would go back home from the farm to eat maize meal, and green leafy vegetables (morogo), and on other days I ate tin fish, other days it was chicken feet, rice, and beef. For supper, I ate maize meal, potatoes, and chicken meat, sometimes with spinach (green leafy vegetables). On other days I ate samp, beetroot, and chicken organs (Participant 20).

I ate 3 meals in a day, I ate breakfast, milk, butter, tea, bread, and cheese. For lunch, I ate maize meal, and beans, tin fish and other day it was samp and beans. For supper, I ate chicken, samp and pumpkin, other days it was cabbage, chicken feet,

beef and maize meal (Participant 19).

*I consumed two times a day, in the morning I consumed maize meal, and green leafy vegetables as my breakfast. Which is the supper food I did not finish the day before because I knew I wouldn't have money for bread. I save a supper meal for the morning. You know maize meal in the early hours of the day can hold your stomach for the whole day, which is better than buying bread every day. During supper, I consumed maize meal and tin fish and the other day I consumed beans and pork (*Participant 16).

The way things are expensive, I consume twice times a day, lunch and supper only. For lunch, I consumed a maize meal, inkomazi, and sometimes a milk and maize meal. On the other days, it was beans, peach from my neighbor's garden, tomato relish, and red meat. For supper, during the past 7 days, I consumed monawa (green leafy vegetable) and maize meal. (Participant 11).

Smallholder farmers' grocery expenditure per month

The data in this study indicated that 54% of the participants in this study spend between R700-R800 monthly on food and groceries that are needed in the household. While 31% of smallholder farmers indicated that between R1200- R1500 monthly is used for household food groceries. Only 15% of smallholder farmers indicated that they purchase R1600- R2200 food every month. It is clear from the smallholder farmers' remarks that a sizeable portion of the population depends on smallholder agriculture for their household's food needs. This data is consistent with that of Ngigi (2011), who discovered that 90% of households in Malawi, 85% of households in Kenya, 76% of households in Botswana, and 75% of households in Zimbabwe are practicing subsistence agriculture to provide their households with nutritious.

Every month end when I do my groceries, I spend between R700-R800 of the money I get from my son. I also supplement it with the one I get from selling the produce (Participant 11).

I spend R1200 most of the time. If I say I spent a lot is when I spent R1500. I don't buy much because I get other food from the farm and I also depend on grants. The money is not enough to buy everything I need at the shop (Participant 19).

*I buy R2000 groceries when I go with my daughter, she chooses almost everything in the shop, giggling, however, I tell her the limit is R2000. If is more should be R2200 (*Participants 18).

Contributions of smallholder farmers toward household food security:

The agricultural sector is regarded as the largest contributor to the economies of most African countries and accounts for over 30% of the continent's Gross Domestic Product (GDP) (Nyange et al. 2011). Agriculture also accounts for a significant percentage of employment, the number of people employed in agriculture, hunting, forestry, and fishing amounted to approximately 868,000 in 2021. This is an increase of 17.3% compared to 2018 (Statista, 2022). In this context, the contributions of smallholder farmers towards household food security have been evaluated under four main headings; increased food supply, job opportunities, and generated income.

Increased food supply

The results of this study indicated that 52% of the smallholder farmers harvest enough from their farms. This is followed by 27% of the smallholder farmers who said they harvest too much from their farms. Additionally, 11% of the smallholder farmers indicated that they harvest little from their farms. This means that smallholder farmers played a critical role in increasing the food supply in some of the households in the study area either as a supplement to the food they bought or solely depending on their farm output. It also means that they have access to food, as they produce more than enough food from their farm. The findings of this study are similar to those of (Matebeni, 2018), who found that agricultural growth can reduce the prevalence of food shortages at all levels through increased overall supply of food for poor people and improved dietary diversity and quality of food consumed by households (Matebeni, 2018).

My harvest is enough because the soil I plant on does not require certain vegetables. I buy other things from the shop. Ya… what I plant and harvest is enough for that time to prevent me from buying other vegetables from the shop which I can plant here and harvest. (Participant 18).

My farm is huge, and the food I produce in every season is so much that I even share it with my neighbours after selling it (Participant 20).

Since I use a small space for crops and another for livestock, my harvest is little, is not much that I can sell. Is only for my family (Participant 11).

Job opportunities

One of the key elements of smallholder production in most rural areas is labor intensity. The data indicate that 55% of the smallholder farmers have large farmland and they further mentioned that they have employed between 8 and 10 people from the local community. While 32% of the smallholder farmers indicated that they have more or less 3 household members helping on the farm during the harvest seasons. It was also found that 13% of the smallholder farmers do not have assistance as they work alone on the farm. Smallholder agriculture has been recognized as one of the most important sectors in employment creation in most developing countries (Baiphethi & Jacobs, 2009). These findings show the importance of smallholder farmers' contribution towards job creation. Many researchers concur with the results of the study that smallholder agriculture creates job opportunities for the local community. As a result, they view the industry as the primary employment source for those without jobs (FAO, 2017).

I have adults whom I work with on the farm every day, there are 8 but sometimes if I need more labourers for the season, I hire an additional 2 workers which makes them 10 in total (Participant 19).

Most of the time I work with my wife and my 2 daughters because my farming space is not big. 3 of us can do a wonderful job on the farm (Participant 11).

On my side, farming is not a big task because my farming area is not big, I work the farming area alone (Participant 20).

Generates income

The data indicated that 56% of the participants generate between R650 and R700 income monthly from selling their harvest, while 41% generate more than R2800-R6000 income monthly from selling their livestock such as pigs, chickens, goats, and cattle. Only 3% of the participants mentioned that they did not generate any income because they did not sell any of their farm output but rather consumed them within their households instead. Smallholder agricultural production is critical for achieving household food security through increased income for the majority of rural poor households. Income generation may provide households with the ability to purchase food from the market to supplement the food they harvested from their farms to meet their food security needs. According to Aref (2011), smallholder farming has largely contributed to rural households' ability to generate money. However, this income was not sufficient, if only they had access to a formal market their income could have been much more than what they receive currently. During the interviews with the participants, they explained as follows:

...after selling the pieces of the slaughtered pig I get about R2800, but if I sell cattle I get R6000. These two animals' prices differ and I can take 6 months without having a customer to come and buy cattle, but with pigs and goats, I get a lot of customers. After selling, that's when I check what I need in the household and buy using the money and also pay debts (Participant 20).

The vegetables and fruits I sell to the community who pass by my farm to buy, in a day I get R650. When I take the products to the street vendors I get 750 a month. I also sell chickens within the community. I sell the slaughtered ones for R70 and not slaughtered by R60 and I get a profit of R2000 (Participant 12).

I don't receive income from my farm produce because I don't sell them (Participant 15).

The challenges faced by the smallholder farmers

Smallholder farmers are faced with various challenges that impede their growth and ability to effectively contribute to food security, compared to commercial farmers. Smallholder farmers' challenges vary from system constraints, and allocation constraints to environmental-demographic constraints (Adebo & Falowo, 2015). Below are the challenges smallholder farmers in Chabelane Village experience.

Water shortages

Water plays a critical role in the success of smallholder agriculture. The data in this study revealed that 87% of smallholder farmers are faced with water shortages. The data further indicated that smallholder farmers use municipal water for irrigation and this water is only available twice a week (Monday and Saturday). Furthermore, the data showed that 11% of the participants have JoJo tanks to save water for irrigation. Only 2% of the smallholder farmers indicated that they irrigate their crops using water from the nearby river, as there is a water problem/shortage in Chabelane Village therefore smallholder farmers find it difficult to irrigate their crops. All of the participants in this study area suffer from a lack of water access/disruption. This is confirmed by Stats SA (2021) stating that 56 % of the general population of Limpopo Province experiences the most water interruptions. Thus, the food production of the smallholder farmers will be affected if this is not attended to by the municipality.

Water is a problem here in Chabelane Village. Most of the time we irrigate using water from the river. The municipality water comes out only on Mondays and Saturdays (Participant 18).

Is quite unfortunate that as farmers we are still faced with water shortages. We use municipality water and we store it in buckets. But when there's no tap water, I struggle to irrigate the crops (Participant 12).

On my farm I have a JoJo tank which I use to store water, but since I have 1 hectare of land for farming. This tank is not enough because sometimes they would tell us that the pipe has burst and they sometimes take two weeks to fix the pipe. This then affects our access to water for irrigation (Participant 19).

Finance challenge

In this study, it was found that all (100%) of the participants have financial challenges. The participants mentioned that there are numerous things that they require to purchase for their farms to operate effectively. But when they try to access different banks, they are denied loans. Participants indicated that they need to purchase farm inputs and pay their laborers, however, financial constraints hamper them. The smallholder farmers in this study showed that they experience a lack of finance to purchase farm inputs, to pay workers, and this negatively impacts their farm production. If banks could approve their loans, it would make a huge difference in their farming to finance their farm input. Opportunity International (2012) asserted that even though most of Africa's population is engaged in agriculture, the continent fails to nourish itself since most of the farmers experience a lack of access to farming inputs or to pay their workers using monetary value due to a shortage of funding. Therefore, for a farming practice to be successful, finance is one of the vital resources that is needed. According to Kuwornu et al. (2012), obtaining agricultural credit promptly may boost smallholder farmers' prospects of obtaining useful assets (such as insecticides, tractors, fertilizer, seeds, and others) that will raise productivity and enhance household's access to food.

The challenge I face now is financial problems. With the little money I get from selling my farm output, I can't buy myself big farm equipment. The banks are denying me loans saying I don't meet their requirement (Participant 16).

Financial issues are one of the huge problems in the farming industry, particularly if you are a black farmer. I depend on grants, and with the little I get from the farm I use to buy seeds and medicines for my livestock. The problem starts when my grandchildren need me to buy them things they need at school. Then I have to take the money I need to buy seeds and assist them. Thereafter, I will not have enough left to maintain my farm (Participant 13).

Lack of access to the formal market

The importance of market access to farmers cannot be overstated. Smallholder farmers experienced a lack of access to formal markets which is a hindrance to their farming success. A lack of sufficient information on how to access/channel their way to the formal market was a hindrance to smallholder farmers of Chabelane Village. When smallholder farmers have access to market information, the transaction costs of the household will be reduced the more the smallholder farmer has market information, which will increase their involvement in the market (Makhura, 2001). Access to a formal market is a problem for smallholder farmers in Chabelane Village, the smallholder farmers indicated that they have good quality vegetables, however, lack of knowledge to access formal market is a challenge. Therefore, information centers on prices from the national point, as well as the type of quality they are looking for should be prioritized for smallholder farmers in the agriculture sector.

I supply to Elim street vendors which is an informal market because I don't know what the formal markets need in terms of requirements to sell to them (Participant 19, 20).

Uhm…what I say is that access to information concerning marketing is a serious issue. As you see me now, I don't know the market price of some of the products I produce today or what is needed the most out there. If we can get assistance or someone to inform us about such knowledge we will be fine, that is my thoughts (Participant 17).

Table 1. The smallholder farmers' practices and smallholder farmers' feedback

Themes	Sub-theme	Responses/data
Smallholder farmers' practices	Type of farming practiced	*Am practicing livestock farming as well as crop farming. I own 15 chickens, 5 cattle, and 6 goats. I plant spinach, beetroot, beans, tomatoes, sweet potatoes, and maize (Participant 7).*
	Reason for farming	*Agriculture is my passion; I was taught by my grandfather how to plant vegetables. Now I practice farming because I want extra income, as for the livestock I inherited them (Participant 6).*

	Farming area and size	*I use my stand to cultivate crops and other space and I have erected two kraals for the livestock (goats and cattle). The farming area is approximately 100 hectares (Participant 8).* *My farming area is about 500 meters square. I practice farming in my yard because the stand is big enough to also plant my crops (Participant 5).*
Household Food prevalence status	Food consumed per day for the past week (7 days)	*During the past week, I ate 3 meals in a day, I ate breakfast, bread, milk, eggs, tea, and butter, and sometimes I ate porridge. Then around 13: 00, I would go back home from the farm to eat maize meal, and green leafy vegetables (morogo), and on other days I ate tin fish, other days it was chicken feet, rice, and beef. For supper, I ate maize meal, potatoes, and chicken meat, sometimes with spinach (green leafy vegetables). On other days I ate samp, beetroot, and chicken organs (Participant 10).* *I had 2 meals in a day in the past 7 days, I ate in the morning maize meal, chicken meat, and green leafy vegetables. I would not finish the supper meal I had the night before; I save some for breakfast because I won't have money for bread for breakfast. You know staple food such as maize meal can hold your stomach for the whole day, which is better than buying bread every day. I also ate oranges from the tree I planted in my yard. During supper, I ate maize meal and cabbage and on other supper days, I ate rice, pumpkin, and fried fish. On Saturday it was chicken feet and maize meal but on Sunday I cooked rice and chicken meat (Participant 8).*
	Smallholder farmers' grocery expenditure per month	*I will estimate because I don't spend the same amount every time, I can say R700. Yeah because I just stay with my 2 grandchildren and my wife only* (Participant 2). *I have a big family, I spend about R2000 on groceries (Participant 4).* *I spend R1000 because I stay with my 2 granddaughters* (Participant 5).
Contributions of smallholder farmers toward household food security	Increased food supply	*I get too much output because I plant too many crops, I do this because I want to supply the street vendors in Gauteng Province as my main priority* (Participant 10). *Most of the time my harvest is little because I would have to discard some of the crops that are not of good quality* (Participant 5). *My harvest is enough and can be supplemented with the food from the shops.* (Participant 1).

	Job opportunities	*Most of the time I call 5 or 6 young boys to come and assist me. They do help me because when it is time to harvest, I have to do so quickly so I can start selling urgently as street vendors require the season stock… What I like about these young boys is they don't demand too much, I just give them some of the harvests as a way of showing appreciation, and sometimes I give my workers money (Participant 5)* *Most of the time I work with my family because I use a small area in my backyard to plant (Participant 1)* *Since I don't have a big area, I cultivate alone even during harvest I do all that alone (Participant 8)*
	Generates income	*The vegetables and fruits I sell to the community who pass by my farm to buy, in a day I get R650. When I take the products to the street vendors, I get 750 a month. I also sell chickens within the community at the wholesale market area using my bakkie. I sell the slaughtered ones for R70 and the un-slaughtered ones for R60, I get a profit of R2000 when I don't count the loss. With the income I make I buy things I need at home, I pay my workers, I buy seeds, and I also save (Participant 5).* *I don't get any income because I don't sell any of my produce. They are for household consumption (Participant 9).*
The challenges faced by the smallholder farmers	Water shortage	*Water is a problem I am facing right now. It does not much in this area. The water I use to irrigate I get from the river near my farm. I use a bucket to store the water and use it to irrigate the crops. I also bring my cattle to graze near this river so that they can have drinking water. Since I know this river, the water doesn't run out (Participant 7)*
	Finance challenge	*The challenge I face now is financial problems. With the little money I get from selling my farm output, I can't buy myself a tractor in 6 months. I need to get at least a loan to add to the one I make. But when I go to banks, they deny me a loan. They tell me that they need something that can convince them that I will be able to repay. Currently, I don't have anything to convince them, unfortunately (Participant 8)*
	Lack of access to the formal market	*I sometimes get good quality spinach, pumpkin, baby marrow, and cabbage, lettuce, tomatoes, the problem is that I don't know how to access those big formal retail stores. I therefore just settled for the community members and street vendors at Mooketsi (Participant 9)*

CONCLUSION AND RECOMMENDATIONS

Consequently, this qualitative research explored the smallholder farmers' contributions towards household food security within Chabelane Village. The study revealed that smallholder farmers in this study are practicing crop farming and livestock farming for household consumption and for selling their produce early for a little extra income. It further indicated that 28% of the smallholder farmers practice farming inside their yards and 63% outside their yards. This study indicated an intricate interplay between smallholder farmers and food security. The findings in this study revealed the prevalence of food security status among the smallholder farmers in Chabelane Village. The majority of the smallholder farmers in this study were found to be food insecure as they did not have enough dietary diversity in a day. Furthermore, the study revealed the contributions of the smallholder farmers towards household food security stemming from the creation of jobs, income generation, and increased food supply. During the interviews, participants highlighted that they come across challenges as smallholder farmers, including water shortages, finance challenges, and lack of access to the formal market. From the challenges mentioned, smallholder farmers find themselves in a serious dilemma to attain food security within their households.

It is important to consider the suggestions found in this study to ensure sustainable food security in Chabelane Village by ensuring the balance between smallholder farmers and food security. These recommendations aim to ensure that smallholder farmers can attain sustainable food security without hindrances that might affect their productivity. Participants had indicated that they experience water interruptions while some participants mentioned that they access water for irrigation from nearby rivers. Greater Letaba Municipality ought to build boreholes for the smallholder farmers and provide them with water tanks for water storage. Participants further indicated that they do not know how to access the formal market, echoing that they end up relying on the informal markets just to survive. Therefore, the Limpopo Department of Agriculture Province should provide an awareness drive for the smallholder farmers in Chabelane Village, to teach them about how to approach the formal market. Moreover, smallholder farmers are challenged by the issue of finances. According to Chimucheka and Rungani (2013), some of the barriers that small enterprises face when trying to obtain financial support include nonexistent financial deposits, shoddy business strategies, inadequate and collateral security. Therefore, for farmers to increase their output and raise household income through the sale of surplus produce, the government should assist them in the form of insecticides, fertilizers, improved seeds, tractors, and pesticides. Additionally, financial organizations like the Land Bank, Commercial Banks, and Micro Agricultural Financial Institutions of South Africa (MAFISA) should assist farmers in rural regions to gain access to credit facilities to increase their output and guarantee food security for their households.

In this context, the study aims to encourage the building of the needed institutional structures and setting in place the basic support pillars such as finance, education, and provision of farm resources that can enable the smallholder farmers to establish their full potential in ensuring food security within their households.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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RESEARCH ARTICLE

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The effect of pH and extraction time on total phenolic content and antioxidant properties of coloured water extracts from *Brassica Oleracea*

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Abstract

Red cabbage (Brassica oleracea) is a rich source of phenolic content including colour pigments and have also high antioxidant capacities. The amounts of their phenolic substances and antioxidant activities change depending on the extraction conditions (pH, time, solvent, etc.). In this work, the coloured water extract was obtained from red cabbage at different pH values (pH 4-10) for both an hour and 24 hours. The extracts were evaluated regarding total phenolic contents and antioxidant activities. The results showed that the total phenolic content of red cabbage extracts in all extraction conditions ranged from 4.93±0.20 to 7.59±1.22 mg GAE/g fw. The highest total phenolic contents (7.59±1.22 mg GAE/g fw) were obtained from red cabbage at 24 h and pH=6. On the other hand, the red cabbage extracts have high DPPH (IC_{50} values ranged from 0.21±0.06 to 0.94±0.03 mg/mL) and ABTS (IC_{50} values ranged from 0.29±0.01 to 0.46±0.05 mg/mL) activities at all pH values and times. The extract obtained from red cabbage at 1 h and pH=7 exhibited the strongest DPPH activity with the IC_{50} values of 0.21±0.06 mg/mL, it showed the best ABTS activity with the IC_{50} values of 0.29±0.01 mg/mL at 1 h and pH=5 and 8.

Keywords: Red cabbage, pH effect, total phenolic content, DPPH Scavenging, ABTS activity

INTRODUCTION

Coloring substances have different chemical structures. Therefore, it has different physical, chemical and physicochemical properties. Naturally occurring colour pigments are important determinants of quality attributes in fresh fruits and vegetables (Stintzing and Carle, 2004). Colorful fruits and vegetables have become very popular today due to the vitamins, minerals, and bioactive substances they contain, and they are recommended to be consumed daily (Amao, 2018). Consumption of fruits and vegetables, as well as nutrition, should be stored in a requirement that should be taken into account due to the evaluation of their importance in human health (Ülger et al., 2018).

Nearly 50 types of vegetables are grown in Türkiye, red cabbage (*Brassica oleracea* L.) has an important place among these vegetables (Karl and Koch, 2013). Red cabbage is a seasonal plant, a type of vegetable that is grown as a red/purple leafy autumn vegetable with large, thick and dark leaf layers. The higher the beta-carotene ratio, the darker the colour (Maltas et al., 2017). Red cabbage gives a red colour in acidic environments and dark blue in basic environments, and it is very important for organic structure that these colours are stable at room temperature. This colour is due to the presence of anthocyanins and is used as a natural food colouring (Chigurupati et al., 2002; Chen et al., 2021). Red cabbage contains a number of bioactive substances such as manganese, potassium, magnesium, calcium, iron and vitamins C, E, K and B6, oligosaccharides, anthocyanins,

flavonols and glucosinolates (Wiczkowski et al., 2013). It helps in the prevention of stomach, lung, intestine, heart diseases and strengthens the immune system. It is known to have anticancer effects (Laje et al., 2010). Moreover red cabbage anthocyanins have received much attention from researchers due to their low cost, availability, abundance, and reliable halochromic capacity (Hosseini et al., 2016; Liang et al., 2019).

The molecular structures of the compounds that give colour to the red cabbage are given in Figure 1.

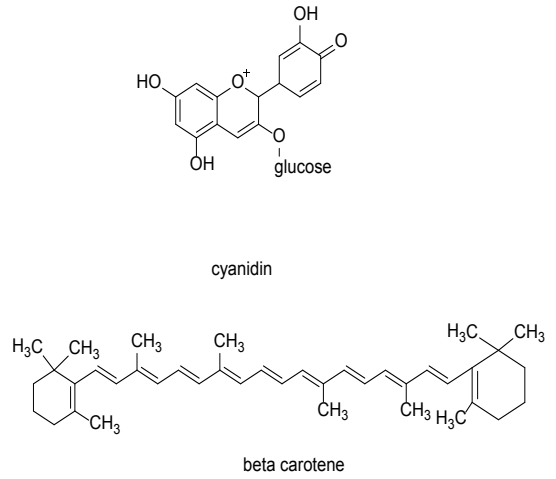

Figure 1. Molecular structure of some colour pigments

In this study, total phenolic compounds were extracted from red cabbage at different pH/different time intervals. The extraction time and pH effects on total phenolic content and ABTS and DPPH scavenging activities as antioxidant properties were investigated.

MATERIALS AND METHODS

Materials

All used chemicals were purchased from Sigma Aldrich and Merck. Red cabbage, grown in Aladağ, Adana (Latitude: 37°32′47″N, Longitude: 35°23′55″E, Türkiye), was sourced from the local market.

Extraction

Red cabbage samples were dissected after cleaning. 10 g sample was extracted with distilled water. The extraction conditions of the samples were adjusted to different pH values (pH 4-10). pH treatment was carried out with citric acid (0.1 N) and sodium carbonate (Na_2CO_3, 10% (w/v)). The extracts were stored in jars in dark at room temperature until their analysis. The color scale of the extracts at different pH values is given in Figure 2.

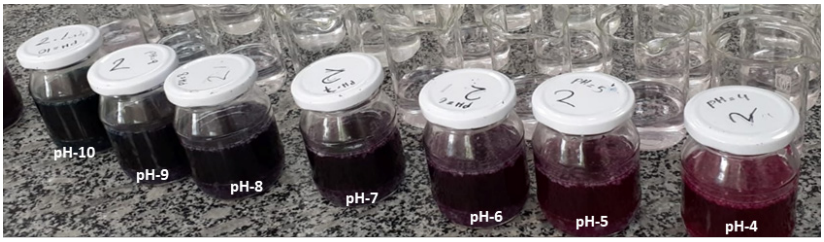

Figure 2. The color scale of the extracts at different pH values.

Total Phenolic Content (TPC)

The total phenolic content of extract was determined by using Folin–Ciocalteu reagent assay (Sonmez and Sahin, 2023). Stock extracts were mixed with Folin-Ciocalteu reagent diluted 1:10 with water. This solution was incubated at 25°C for 3 min. and mixed with 1 mL of Na_2CO_3. The absorbance value was measured at 765 nm after 60 min. of incubation in the dark. A standard curve with gallic acid was drawn. The total amount of phenolic compounds was calculated and expressed as mg GAE (Gallic Acid Equivalent) /g fw (fresh weight).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of extract was measured according to the method described by Sonmez et al.

(Sonmez et al., 2019). Samples at different concentrations were prepared from extracts with different pH values. 3 mL of DPPH solution was mixed with these different concentrations of the extracts. This thoroughly mixed solution was incubated for 30 minutes. After incubation, absorbance values were determined at 517 nm wavelength. IC_{50} values are calculated by from the graph of %inhibition against the extract concentration.

% DPPH inhibition = $[(Abs_c - Abs_s) / Abs_c]*100$

ABTS Radical Scavenging Activity

ABTS scavenging activities of the extract were measured according to the method described by Sonmez et al. (Sonmez et al., 2022). The solution of ABTS radical was generated by dissolving ABTS and $K_2S_2O_3$ in distilled water. This solution was kept in dark for 18-24 h at room temperature. The absorbance of this prepared solution was adjusted to be 0.70±0.01 at 734 nm. It was mixed with ABTS prepared with samples of different concentrations. The absorbance of the samples was measured at 734 nm 6 min. after mixing. The change in absorption was used for calculation of activity.

Statistical analysis

The data are presented as mean ±SD for triplicate analysis. Statistical evaluations were analysed by Minitab Statistical Software using ANOVA with a 95% confidence interval.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity values of red cabbage are presented in Table 1.

The results showed that total phenolic contents (TPC) of red cabbage extract is between 4.93± 0.2 and 6.48± 0.77 mg GAE/g fw at pH=4-10 and 1h. The total phenolic content of red cabbage extract is between 5.64± 0.16 and 7.59± 1.22 mg GAE/g fw at pH=4-10 and 24h. Red cabbage extract had the highest total phenolic content at 24h and pH=6. Generally, increasing extraction time raised the TPC of red cabbage. In the study conducted by Hunaefi (2013) on red cabbage, its total phenolic content was stated as (175.89 ± 15.99 mg GAE.100g fw^{-1}).Moreover the total phenolic content of four common vegetables was determined by Djeussi et al. (2022) and red cabbage was reported as 4.37±0.32 mg of gallic acid/ gram of dried extract. It was observed that all of the total phenolic content measured at different pH and different times were higher than the value reported by Hunaefi et al. and by Djeussi et al.

Red cabbage extracts showed DPPH scavenging activity with an IC_{50} value of 0.21± 0.06 - 0.34± 0.01 mg/mL at 1h and with an IC_{50} value of 0.34±0.01 - 0.94±0.03 mg/mL at 24h. Among them, red cabbage extract exhibited the strongest DPPH scavenging activity at 1h and pH=7. The antioxidant activity of fresh and dried red cabbage was examined by Efendi et al (2022). The antioxidant activity IC_{50} value of fresh and dried red cabbage was reported as 54.317 ppm and 49.464 ppm, respectively. In addition to the antioxidant properties of red cabbage in different solvents were stated by Önder et al. (2020), and IC_{50} values were determined as 63±1.41 µg/mL in the ethenol:water extract and 60±1.41 µg/mL in the methanol extract. The antioxidant effect of the extracts obtained in this study is much stronger than the two reported studies.

Red cabbage extracts showed ABTS activity with an IC_{50} value of 0.29± 0.01 - 0.38± 0.02 mg/mL at 1h, and with an IC_{50} value of 0.33±0.01 - 0.46±0.05 mg/mL at 24 h. Among them, red cabbage extract exhibited the highest ABTS activity at 1h and pH=5 and pH=8.

Table 1. Total phenolic content (TPC), DPPH scavenging activity and ABTS activity (IC_{50} values) of red cabbage extracts obtained for 1h and 24 h.

pH	Sample	TPC (mg GAE/ g fw)		DPPH (IC_{50}, mg/mL)		ABTS (IC_{50}, mg/mL)	
		Extraction time					
		1h	24 h	1h	24 h	1h	24 h
4	red cabbage	6.48±0.77^{a}	7.01±0.24^{a,b}	0.32±0.08^{a}	0.34±0.01^{d}	0.32±0.03^{c}	0.46±0.05^{a}
5	red cabbage	5.66±0.22^{b}	6.99±0.54^{a,b}	0.34±0.01^{a}	0.39±0.06^{c,d}	0.29±0.01^{d}	0.33±0.01^{b}
6	red cabbage	5.37±0.09^{c}	7.59±1.22^{a}	0.26±0.02^{b}	0.46±0.16^{b,c,d}	0.32±0.02^{b,c}	0.38±0.03^{b}
7	red cabbage	5.37±0.14^{c}	5.95±0.42^{c}	0.21±0.06^{c}	0.67±0.14^{a,b,c}	0.32±0.09^{d}	0.35±0.01^{b}
8	red cabbage	5.62±0.40^{b}	6.36±0.20^{c,b}	0.28±0.08^{a,b}	0.72±0.18^{a,b}	0.29±0.11^{d}	0.33±0.01^{b}
9	red cabbage	4.94±0.22^{d}	6.28±0.10^{c,b}	0.25±0.11^{b}	0.94±0.03^{a}	0.34±0.08^{b}	0.34±0.01^{b}
10	red cabbage	4.93±0.20^{d}	5.64±0.16^{c}	0.25±0.06^{b}	0.89±0.83^{a}	0.38±0.02^{a}	0.37±0.02^{b}

Results are expressed as means ± SD (standard deviation) (n=3). a-d Refers the significant differences between the values in the same column ($P < 0.05$).

Generally, red cabbage extract has high TPC and also stronger DPPH and ABTS activities both extraction time and all pH values. The changing extraction time and pH did not linearly affect DPPH and ABTS activities of this extract.

CONCLUSIONS

In conclusion, red cabbage extracts, obtained different pH values and extraction time, were compared in terms of total phenolic content and antioxidant properties. The results showed that red cabbage had a significantly high phenolic content. Moreover, the red cabbage extracts showed strong antioxidant activity. It was determined that the extraction time is 1h and pH value is 6 for the highest TPC of red cabbage extract, while extraction time is 1h and pH value is 7 for the strongest DPPH activity. On the other hand, it was observed that extraction time is 1h, pH values are 5 and 8 as the best conditions for ABTS activity. Total phenolic content, DPPH and ABTS antioxidant activity values of the samples extracted under different pH conditions were found to be statistically significant ($p<0.05$). According to all these results, it is considered that red cabbage extracts may be preferred to use for as natural colorant additive for various nutrition.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

Not applicable

Consent for publication

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RESEARCH ARTICLE

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Safety of agricultural machinery and tractor maintenance planning with fuzzy logic and MCDM for agricultural productivity

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Abstract

Productivity is one of the most important measures used to determine the growth and development level of countries or sectors. A wide variety of projects have been planned and implemented to increase agricultural productivity. The productivity to be obtained in agriculture; Soil conditions, climate, seeds, fertilizer, pesticides, labor and agricultural mechanization directly affect it. Agricultural mechanization is the realization of agricultural activities by using energy together with agricultural tools and machines. Agricultural mechanization; It is an important agricultural production technology that helps increase agricultural productivity. Due to the inadequate maintenance planning of agricultural machinery, agricultural machinery cannot be utilized at the desired level in agricultural production. Most agricultural equipment is subject to frequent changes in speed and direction of movement while operating. Damage that can be seen on a single machine; It also causes other machines to malfunction. During the year, especially in the months when agricultural activity is high, excessive working tempo can cause tractors to malfunction. The breakdown of tractors causes disruptions in agricultural activities. In addition, the breakdown of tractors increases the repair costs. Since there is no tractor maintenance planning, farmers face interruptions in agricultural activities due to tractor malfunction. However, tractor malfunctions may cause cost and economic losses. For these reasons, there is a need for appropriate maintenance planning of agricultural machinery in order to continue agricultural activities without disruption. Maintenance planning; It consists of a set of preventive activities to improve the reliability and availability of any system. The main purpose of this study is to determine and rank the importance level weights of the criteria that are important for agricultural machinery maintenance planning using the fuzzy AHP method. Fuzzy AHP method, which provides ease of application, was preferred in determining the Criterion Weights. The research proposes a framework to determine the weights of appropriate criteria for care planning selection through a combined approach of fuzzy multi-criteria decision making involving relevant stakeholders. On the basis of the prioritization of criteria of tractor maintenance planning (TMP), it was found from the ranking that checking for all fluid levels (TMP1) ranked first. This respectively is followed by checking for general conditions (TMP4), checking for tires and wheels (TMP2) and checking for batteries (TMP3). With the results of the study, a guide was created for farmers and other stakeholders, as well as decision makers, to help plan the maintenance of machines in better working conditions. It is also thought that this study will be encouraging for other studies.

Keywords: Agricultural Marketing, Agricultural Productivity, Agricultural Mechanization, MCDM, fuzzy AHP

INTRODUCTION

Productivity is one of the most important measures used to determine the growth and development level of countries or sectors. Economic growth and development can be achieved by including idle resources into production and directing currently used resources to more productive areas (Bayramoglu, 2010). This also represents an increase in productivity. Productivity; It can be defined as the ratio between the amount of goods and services produced and the inputs used to produce this amount of goods and services. A wide variety of projects have been planned and implemented to increase agricultural productivity. Moreover, academic research on these subjects supports this (Table 1). The productivity to be obtained in agriculture; Soil conditions, climate, seeds, fertilizer, pesticides, labor and agricultural mechanization directly affect it. It is a fact that especially with the introduction of tractors into field work, it provides great convenience to farmers in every aspect (Dogan, 2012). The agricultural sector is negatively affected by global market instability, economic crisis, animal diseases and climate changes in recent years (Ozguven et al., 2010). However, structural problems in the agricultural sector cause in inproductivity. In order to carry out agricultural activities in an optimum way and to obtain a good crop as a result, it is inevitable to use some inputs (Isik et al., 2003). Productivity increase is possible by using inputs (fertilizer, pesticides, seeds) in harmony with each other (Ozguven et al., 2010).

Agricultural mechanization is the realization of agricultural activities by using energy together with agricultural tools and machines. Agricultural mechanization; It is an important agricultural production technology that helps increase agricultural productivity. According to the Food and Agriculture Organization of the United Nations (FAO, 2023), sustainable mechanization contributes to the sustainable development of the food and agriculture sector; It takes into account technological, economic, social, environmental and cultural dimensions. The use of machinery in agriculture, unlike other agricultural technology applications, indirectly affects the increase in productivity; It enables the application of new production methods in rural areas (Ozguven et al., 2010). Technological developments in agriculture have increased the importance of mechanization and enabled more productivity per unit area in agricultural production (Altuntas, 2016). Agricultural mechanization in agricultural enterprises is implemented at different levels depending on the technical and economic structure of the enterprise (Zeren et al., 1995). The level of agricultural mechanization must be planned correctly in order to meet the rapidly increasing demand for agricultural crops, increase the current production level and increase productivity. The level of mechanization may have different values in each agricultural enterprise, depending on the technical and economic structure of the enterprise (Kocturk and Avcioglu, 2007). Planning the level of agricultural mechanization; This can be achieved by increasing the diversity of the tractor and agricultural equipment-machinery park and making it more effective (Altuntas and Demirtola, 2004). Due to the inadequate maintenance planning of agricultural machinery, agricultural machinery cannot be utilized at the desired level in agricultural production. Agricultural mechanization can achieve its goal with the availability of appropriate and sufficient equipment for the tractor, which is the main power source in agriculture (Altuntas and Demirtola, 2004). The use of agricultural mechanization increases the productivity of the workforce and the productivity of the use of other resources (Oguz et al., 2017). As a result of unplanned mechanization, the balance between agriculture and industry may be disrupted to the detriment of agriculture and may lead to an increase in unemployment in rural areas (Ozguven et al., 2010). Increasing agricultural mechanization can only be achieved by correct agricultural mechanization planning (Toga, 2006).

Most agricultural equipment is subject to frequent changes in speed and direction of movement while operating. In addition, the operating conditions of these devices; It also varies greatly due to agricultural activity and environmental factors. Due to all these factors, agricultural machinery is exposed to various loads and causes damages (Mishra and Satapathy, 2023). Damage that can be seen on a single machine; It also causes other machines to malfunction. In the studies, maintenance planning problems have been discussed from different angles in different sectors (Barabady and Kumar, 2008; Gu and Huang, 2010; Bose et al., 2012; Jurca, 2012; Poozesh et al., 2012; Lynch et al., 2013; Khodabakhshian, 2013; Afsharnia et al., 2014; Amini Khoshalan et al., 2015; Najafi et al., 2015; Obinna and Oluka, 2016; Wolfert et al., 2017; Rybacki and Grześ, 2018; Da Silva et al., 2019).

The tractor, which is the most important form of mechanization in agriculture, is an important tool for agricultural statistics. However, the agricultural development function of a country is evaluated by the presence and abundance of tractors (Dogan, 2012). Tractors are very important for agricultural activities. With developing technology, more modern and more functional tractors; It contributes to agricultural work with a wide variety of functions (Dogan, 2012). During the year, especially in the months when agricultural activity is high, excessive working tempo can cause tractors to malfunction. The breakdown of tractors causes disruptions in agricultural activities. Disruptions in agricultural activities; It may cause inefficient use of labor and losses in production (Mishra and Satapathy, 2023). In addition, the breakdown of tractors increases the repair costs. Especially in developing countries; Lack of adequate preventive maintenance planning increases the problem of tractor malfunctions due to spare parts shortage and similar reasons. Since there is no tractor maintenance planning, farmers face interruptions in agricultural activities

due to tractor malfunction. However, tractor malfunctions may cause cost and economic losses. For these reasons, there is a need for appropriate maintenance planning of agricultural machinery so that agricultural activities can be continued without disruption (Mishra and Satapathy, 2023). Maintenance planning; It consists of a set of preventive activities to improve the reliability and availability of any system. Studies on the analysis of machine malfunctions have focused mostly on maintenance, malfunction risks, malfunction probabilities and malfunction detection (Table 1). In many studies where uncertainty is taken into account, MCDM methods are applied to solve different problems (Atlı, 2024). Although many studies have been conducted in which uncertainty is taken into account using MCDM methods, the number of studies applied to agricultural sectors is quite limited. The studies are mostly in the agricultural supply chain; strategies, supplier selection, location selection, strategy selection and planning (Kaviani et al., 2020; Lau et al., 2020; Rani et al., 2020; Ozkan et al., 2020; Durczak et al., 2020; Mugiyo et al., 2021; Ronaghi and Mosakhani, 2022; Yazdani et al., 2022).

When the studies are examined, it is seen that different techniques and criteria are applied as evaluation tools. Generally, expert opinion is needed to evaluate the current situation in order to decide which criteria among the proposed criteria will be the most appropriate. The selection of evaluation criteria is the most important part of the care planning evaluation. In this study, it was first aimed to determine the criteria that could be effective in maintenance planning. For this purpose, the criteria frequently used in the literature for the selection of maintenance planning were examined in a meeting environment with experts who have experience in maintenance planning, and the criteria that were thought to be the most important for the sector were evaluated. In this study, the criteria considered to be the most important were applied, adapted from the study of Mishra and Satapathy (2023). Criteria and sub-criteria are shown in (Figure 4).

This research contributes to the literature in the following ways: It is the first study in which the maintenance planning of agricultural machinery is applied with fuzzy AHP for agricultural productivity in the agricultural sector. However, there are modeling studies that examine the maintenance planning selection problem on a sector-by-sector basis using classical MCDM methods. The criteria were adapted from the study of Mishra and Satapathy (2023) in order to make a general evaluation of maintenance planning in the agricultural sector, taking into account the opinions of decision makers. Fuzzy AHP method, which provides ease of application, was preferred in determining the Criterion Weights. The research proposes a framework to determine the weights of appropriate criteria for care planning selection through a combined approach of fuzzy multi-criteria decision making involving relevant stakeholders.

The main purpose of this study is to determine and rank the importance level weights of the criteria that are important for agricultural machinery maintenance planning using the fuzzy AHP method. Fuzzy AHP method, which provides ease of application, was preferred in determining the Criterion Weights. The research proposes a framework to determine the weights of appropriate criteria for care planning selection through a combined approach of fuzzy multi-criteria decision making involving relevant stakeholders. In this study, first of all, a comprehensive analysis of the literature in the field was made. A wide variety of elements have been identified and classified related to the basic elements of agricultural productivity, agricultural mechanization, maintenance planning and agricultural machinery safety. Then, the method and application are given. Finally, the results of the study are summarized.

MATERIALS AND METHODS

The main purpose of this study is to determine and rank the importance level weights of the criteria that are important for agricultural machinery maintenance planning using the fuzzy AHP method. With the results of the study, a guide was created for decision makers, farmers and other stakeholders to help plan the maintenance of machines in better working conditions. It is also thought that this study will be encouraging for other studies. For agricultural machinery maintenance planning, decision makers have the task of identifying potential criteria that will complement that decision-making process. The MCDM flowchart for agricultural machinery maintenance planning is shown in (Figure 1).

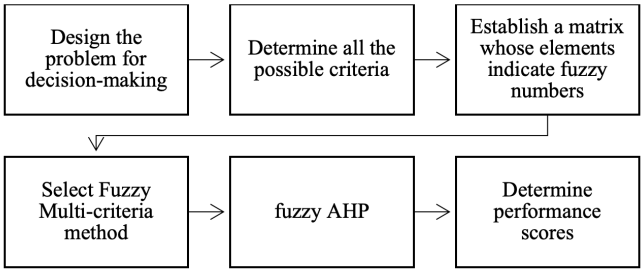

Figure 1. MCDM flowchart for agricultural machinery maintenance planning.

Table 1. Agricultural mechanization and agricultural productivity literature.

Autor	Research Purpose
Irz et al., 2001; Olesen and Bindi, 2002; Ruttan, 2002; Gollin et al., 2002; Wiebe, 2003; Thirtle et al., 2003; Goldsmith et al., 2004; Robertson and Swinton, 2005; Coelli and Rao, 2005; Lee, 2005; Dale and Polasky, 2007; Molden et al., 2007; Li et al., 2007; Restuccia et al., 2008; Mittal and Tripathi, 2009; Mittal et al., 2010; Molden et al., 2010; Alston et al., 2010; O'Donnell, 2010; Gornall et al., 2010; Gollin, 2010; Burney et al., 2010; McMillan and Rodrik, 2011; Connor et al., 2011; Peterman et al., 2011; Chand et al., 2011; Davis et al., 2012; Savci, 2012; Lobell and Gourdji, 2012; Cassidy et al., 2013; Reimers and Klasen, 2013; Lagakos and Waugh, 2013; Kurukulasuriya and Rosenthal, 2013; Teklewold et al., 2013; Adamopoulos and Restuccia, 2014; Gollin et al., 2014; Alston and Pardey, 2014; Kilic et al., 2015; Emerick et al., 2016; Bustos et al., 2016; Lawry et al., 2017; Adamopoulos and Restuccia, 2020; Ortiz-Bobea et al., 2021.	Agricultural productivity
Martin and Olmstead, 1985; Binswanger, 1986; Pingali et al., 1987; Clarke, 2000; Pingali, 2007; Kocturk and Avcioglu, 2007; Asoegwu and Asoegwu, 2007; Ozguven et al., 2010; Houmy et al., 2013; Houssou et al., 2013; Akinbamowo, 2013; Yang et al., 2013; Takeshima et al., 2013; Benin, 2015; Iqbal, 2015; Biggs and Justice, 2015; Amare and Endalew, 2016; Luo et al., 2016; Diao et al., 2016; Sims and Kienzle, 2017; Li et al., 2018; Mrema et al., 2018; Emami et al., 2018; Aryal et al., 2019; Jiang et al., 2020; Van Loon et al., 2020; Takeshima et al., 2020; Daum and Birner, 2020; Belton et al., 2021; Qian et al., 2022.	Agricultural mechanization
Paman et al., 2010; Fathollahzadeh et al., 2010; Rohani et al., 2011; Mousazadeh et al., 2011; Spinelli et al., 2011; Moorehead et al., 2012; Vernon and Meier, 2012; Lips and Burose, 2012; Khodabakhshian, 2013; Lorencowicz and Uziak, 2015; Baudron et al., 2015; Takeshima et al., 2015; Pickett et al., 2015; Redreev, 2016; Mantoam et al., 2016; Redreev et al., 2017; Myalo et al., 2018; Galiev et al., 2018; Redreev et al., 2018; Gupta et al., 2019; Hrytsaienko et al., 2019; Myalo et al., 2019; Redreev et al., 2020; Galiev et al., 2020; Elhaki and Shojaei, 2020; de Araujo Zanella et al., 2020; Daum et al., 2021.	Tractor maintenance

Selection of the best maintenance alternative in maintenance planning of machines, selection of the most appropriate multi-criteria decision-making (MCDM) model to evaluate each alternative according to a set of criteria; It is a multi-criteria decision making problem. In the methodology section, fuzzy numbers and fuzzy AHP used in the study and its application steps are given. Additionally, the scales used to convert numbers into fuzzy ones are presented. In this study, the importance levels and weights of the evaluation criteria were measured by the fuzzy AHP method. Accordingly, the rankings of the main criteria and sub-criteria were obtained.

Fuzzy Logic and Fuzzy Numbers

Fuzzy logic is a logic structure formed by the article "fuzzy sets and systems" published by Zadeh (1965) and the article "fuzzy logic and approximate reasoning" by Zadeh (1975). Fuzzy sets, basic operations, concepts and properties are

given in this article. According to Zadeh (2015: 4), one of the main contributions of fuzzy logic is to provide a basis for progress from binarization to gradation, from binary to pluralism, from black and white to shades of grey. Fuzzy logic theory offers a number of methods and rules that take into account the uncertainty, indecision and imprecision in verbal expressions and express them numerically. According to Sergi (2021), such imprecise linguistic terms, which are quite suitable for the human mindset, are used in people's decision-making mechanism in the face of an event or situation.

The basis of fuzzy logic is based on fuzzy sets and subsets. In the fuzzy set, each object has a degree of membership. A fuzzy set is a set of objects with a continuous degree of membership (Ertugrul, 2007: 175). The fuzzy set characterizes each object with a membership function with a membership degree varying between 0 and 1 (Zadeh, 1965: 338). There are membership functions in different forms that define fuzzy sets analytically and represent their membership degrees, and the most commonly used among the various forms of fuzzy membership functions are triangular, trapezoidal, Gaussian and generalized bell curve membership functions (Sergi, 2021: 56). In this study, triangular fuzzy numbers were used. To create the decision matrix, linguistic expressions were transformed into triangular fuzzy number form.

Triangular fuzzy numbers were created to maximize the accuracy of the evaluations in uncertain evaluations when making decisions (Arslankaya and Göraltay, 2019:56). Parameters expressed as (*l, m, u*) show the smallest possible value, the most probable value and the largest possible value, respectively (Ertugrul, 2007). Equation (1) is given in (Hudec, 2016), and the graph drawn for the function is given in (Figure 2). The membership function of the triangular fuzzy number is defined as follows (Equation 1):

$$\mu_{\tilde{A}}(x) = \begin{cases} 0, & if\ x \le l \\ \dfrac{x-l}{m-l}, & if\ l \le x \le m \\ \dfrac{u-x}{u-m}, & if\ m \le x \le u \\ 0, & if\ u \le x \end{cases} \qquad (1)$$

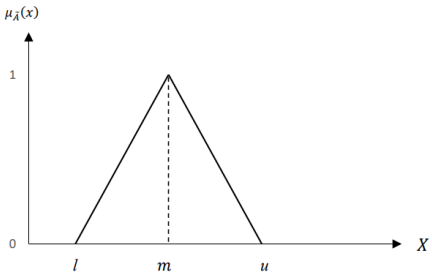

Figure 2. Triangle Membership Function.

Calculation of criterion weights with the fuzzy AHP method

Analytic Hierarchy Process (AHP) was first proposed by Myers and Alpert (1968). Analytic Hierarchy Process (AHP) is a multi-criteria decision-making method based on pairwise comparison developed by Thomas L. Saaty (1977 and 1982) for the solution of complex measurement and decision-making problems involving a large number of criteria and alternatives. Method; It offers a hierarchical structure that expresses the connection between the purpose of the problem, its criteria and alternatives. The AHP approach is used effectively in the decision-making process in many real-life problems (Ustalı and Tosun, 2019). Since it is not sufficient to evaluate situations of uncertainty and imprecision (Deng, 1999); The AHP method was combined with fuzzy logic and the fuzzy AHP approach started to be used as a new method. There are many studies based on B-AHP techniques in the literature (Chan and Kumar, 2007; Subramanian and Ramanathan, 2012; Xu and Liao, 2013; Ghadikolaei and Esbouei, 2014; Keršulienė and Turskis, 2014a, 2014b; Nguyen et al., 2015; Turskis et al., 2015; Mavi, 2015; Zavadskas et al., 2015; Shafiee, 2015; Prakash and Barua, 2016; RazaviToosi and Samani, 2016; Wang et al., 2016; Kubler et al., 2016; Soberi and Ahmad, 2016; Nguyen et al., 2016; Emrouznejad and Marra, 2017; Turskis et al., 2019; Liu et al., 2020; Wang et al., 2021; Fu et al., 2021; Bakır and Atalık, 2021; Atlı, 2022).

In the structure of the AHP method; First of all, there is the decision maker, this decision maker has a goal/target set and has many alternatives to choose or rank. Of course, the criteria to be used in evaluating these alternatives and the weights of these criteria should be determined. As a result, a "decision matrix" should be created using these values and implemented (Eren, 2021). There are many fuzzy AHP application methods in the literature. In this study, the B-AHP application method, which is more practical and easier to apply, was used. The application steps of the B-AHP approach are as follows (Soberi and Ahmad, 2016; Atlı, 2022):

Creating the Hierarchical Structure

The hierarchy consists of different levels that allow decision makers to view their problems from a comprehensive framework, ranging from the purpose of the problem to a set of various criteria and alternatives. A hierarchical model was created containing the research problem, 4 main criteria and 16 subcriteria. The hierarchy created for the research problem is shown in (Figure 3).

Pairwise comparison matrices between criteria

After the hierarchical structure is created, binary comparison matrices are created in line with the opinions of the decision makers (Equation 2). In cases where there is more than one decision maker, the pairwise comparison matrices created by the decision makers are converted into a combined pairwise comparison matrix.

$$\tilde{A} = \begin{bmatrix} 1 & \tilde{a}_{12} & \dots & \tilde{a}_{1n} \\ \tilde{a}_{21} & 1 & \dots & \tilde{a}_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \tilde{a}_{n1} & \tilde{a}_{n2} & \dots & 1 \end{bmatrix} = \begin{bmatrix} 1 & \tilde{a}_{12} & \dots & \tilde{a}_{1n} \\ 1/\tilde{a}_{12} & 1 & \dots & \tilde{a}_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ 1/\tilde{a}_{1n} & 1/\tilde{a}_{2n} & \dots & 1 \end{bmatrix}$$

$$\tilde{a}_{ij} = \begin{cases} \tilde{1}, \tilde{3}, \tilde{5}, \tilde{7}, \tilde{9} & Criterion\ i\ is\ more\ important\ than\ criterion\ j \\ 1, & i = j \\ \tilde{1}^{-1}, \tilde{3}^{-1}, \tilde{5}^{-1}, \tilde{7}^{-1}, \tilde{9}^{-1} & Criterion\ i\ is\ less\ important\ than\ criterion\ j \end{cases} \quad (2)$$

Fuzzy triangular scales are used to determine the priorities of criteria in the hierarchy, reflecting the relative importance among other criteria (Soberi and Ahmad, 2016). When decision makers evaluate criteria, they are compared using linguistic terms as shown in (Table 2).

Table 2. Linguistic terms and the corresponding triangular fuzzy scale.

Linguistic terms	Fuzzy Triangular Scale	Triangular Fuzzy Correspondence Scale	Saaty scale
Equally important (Eq. Imp.)	(1,1,1)	(1,1,1)	1
Weakly important (W. Imp.)	(1,3,5)	(1/5,1/3,1/1)	3
Fairly important (F. Imp.)	(3,5,7)	(1/7,1/5,1/3)	5
Strongly important (S. Imp.)	(5,7,9)	(1/9,1/7,1/5)	7
Absolutely important (A. Imp.)	(7,9,9)	(1/9,1/9,1/7)	9

Sources: Chang (1996); Atlı (2022).

Pairwise comparisons between all criteria are made by decision makers. Pairwise comparisons made by decision makers are combined by taking the geometric mean of the collected data suggested by Saaty and a common opinion is obtained. The reason why the geometric mean is preferred to the arithmetic mean method is that it satisfies the rule that symmetric elements in the comparison matrix must be inverse of each other (Omurbek and Tunca, 2013). The pairwise comparison data of each criterion in the triangular fuzzy scale in (Table 2) are then synthesized into matrix contribution form.

Normalized relative weights of criteria

In creating the dual pairwise comparison matrix, fuzzy geometric means and fuzzy weights of each criterion are determined by using the geometric mean method of Buckley (1985). In this step, the $\tilde{r}_i$ fuzzy comparison value is found using Equation (3). Then, the geometric mean of the fuzzy comparison value is taken.

$$\tilde{r}_i = \left(\prod_{j=1}^{n} \tilde{d}_{ij} \right)^{1/n} \quad (3)$$

The geometric means of fuzzy values is then converted to relative fuzzy of weight by multiplying them with the total of reverse fuzzy geometric means in increasing order by using Equation (4).

$$\tilde{w}_i = \tilde{r}_i \otimes [\tilde{r}_1 \oplus \dots \oplus \tilde{r}_i \oplus \dots \tilde{r}_n]^{-1} \quad (4)$$

Finally, the relative non-fuzzy weight of each criteria is calculated by averaging the fuzzy numbers for each criteria. The normalized weights of each criteria, is calculated by dividing the each value of relative fuzzy weight with the total of all criteria's value.

RESULTS AND DISCUSSION

Calculation of criterion weights with the fuzzy AHP method

Creating the Hierarchical Structure

A hierarchical model has been created that allows decision makers to enter their problems from a comprehensive framework and includes the purpose of the problem, 4 main criteria and 16 subcriteria. The hierarchy created for the research problem is shown in (Figure 3).

Pairwise comparison matrices between criteria

To create the pairwise comparison matrix, nine experts were interviewed to compare the criteria using the B-AHP method. Experts were asked to make pairwise comparisons of the criteria according to the B-AHP scale (Chang, 1996; Atlı, 2022) shown in (Table 2). Pairwise comparisons between all criteria were made by decision makers. A common opinion was obtained by combining the pairwise comparisons made by the ground transmitters by taking the geometric mean of the collected data suggested by Saaty. The pairwise comparison data of each criterion relative to each other on a triangular scale from (Table 2) were then synthesized into matrix contribution form.

Data on performance values of the criteria were received from decision makers. The evaluations of decision makers were transformed into triangular fuzzy numbers through linguistic variables and the combined values are given in (Tables 3, 4, 5, 6, 7).

Normalized relative weights of criteria

In creating the dual pairwise comparison matrix, fuzzy geometric means and fuzzy weights of each criterion were determined by using the geometric mean method of Buckley (1985). In this step, the fuzzy comparison value was found using Equation (3) (Tables 8, 9, 10, 11, 12). Then, the geometric mean of the fuzzy comparison value was taken.

Then, the geometric means of the fuzzy values were converted to relative weight blur by multiplying them with the sum of the inverse fuzzy geometric means in increasing order using Equation (4) (Table 13). Finally, the relative non-fuzzy weight of each criteria was calculated by averaging the fuzzy numbers for each criteria. The normalized weights of each criteria, were calculated by dividing the each value of relative fuzzy weight with the total of all criteria's value (Table 14, 15). The ranking of criteria and subcriteria according to their global weights with fuzzy AHP is shown in (Table 16). On the basis of the prioritization of criteria of tractor maintenance planning (TMP), it was found from the ranking that checking for all fluid levels (TMP1) ranked first. This respectively is followed by checking for general conditions (TMP4), checking for tires and wheels (TMP2) and checking for batteries (TMP3). Identifying all errors and error mechanisms of a system; It is an analysis to evaluate the effects of each potential error on system security and performance and to classify each error according to its criticality (Cekel and Acar, 2023). The points that should be included in tractor maintenance planning should be determined according to the critical functions of the vehicle that affect its mission. Cekel and Acar (2023) made failure modes and criticality analyzes by choosing the engine subsystem as the subsystems that are mostly handled in periodic maintenance applications of the tractor. However, with good tractor maintenance planning, risks and accidents can be prevented by foreseeing error possibilities. If tractor maintenance planning is not implemented, it increases risks and accidents. For example; Reasons such as the failure of the brake system to function due to neglect of the tractor and the brakes not working when the brakes are applied frequently have been effective in the risk of accidents (Erdal, 2005; Yildirim and Altuntas, 2015).

Table 3. Combined comparison matrix (Main criteria).

CRI		TMP1			TMP2			TMP3			TMP4	
TMP1	1,000	1,000	1,000	2,485	3,195	3,475	3,637	6,031	7,770	2,432	3,482	5,196
TMP2	0,288	0,313	0,402	1,000	1,000	1,000	4,787	6,853	8,452	0,192	0,270	0,378
TMP3	0,129	0,166	0,275	0,118	0,146	0,209	1,000	1,000	1,000	0,129	0,166	0,275
TMP4	0,192	0,287	0,411	2,646	3,708	5,196	3,637	6,031	7,770	1,000	1,000	1,000

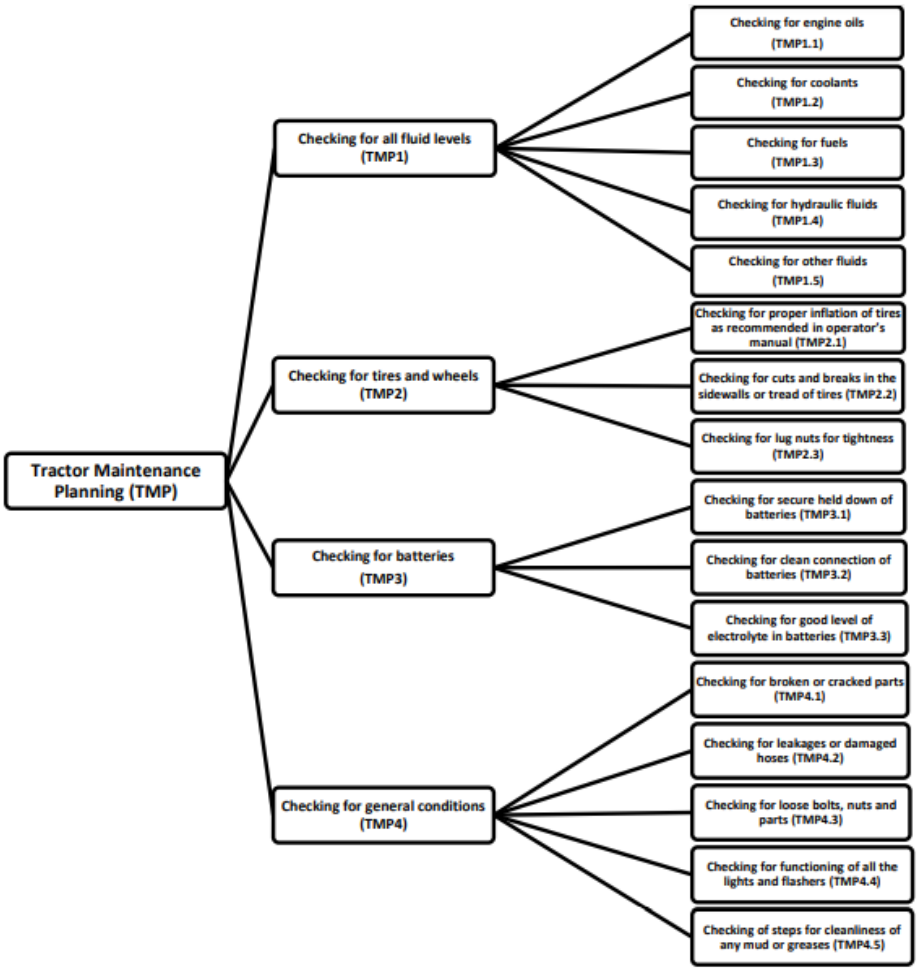

Figure 3. Hierarchical model.

Table 4. Combined comparison matrix (TMP1 subcriteria).

CRI	TMP1.1			TMP1.2			TMP1.3			TMP1.4			TMP1.5		
TMP1.1	1,000	1,000	1,000	3,956	6,422	7,770	4,787	6,853	8,452	3,956	6,422	7,770	3,637	6,031	7,770
TMP1.2	0,129	0,156	0,253	1,000	1,000	1,000	3,201	5,544	7,297	1,769	3,000	4,486	0,577	0,880	1,210
TMP1.3	0,118	0,146	0,209	0,137	0,180	0,312	1,000	1,000	1,000	0,386	0,508	0,809	0,137	0,180	0,312
TMP1.4	0,129	0,156	0,253	0,223	0,333	0,565	1,236	1,968	2,590	1,000	1,000	1,000	0,333	0,467	0,615
TMP1.5	0,129	0,166	0,275	0,827	1,136	1,732	3,201	5,544	7,297	1,627	2,141	3,000	1,000	1,000	1,000

Table 5. Combined comparison matrix (TMP2 subcriteria).

CRI	TMP2.1			TMP2.2			TMP2.3		
TMP2.1	1,000	1,000	1,000	1,495	2,010	3,000	0,223	0,270	0,411
TMP2.2	0,333	0,497	0,669	1,000	1,000	1,000	0,863	1,236	1,592
TMP2.3	2,432	3,708	4,486	0,628	0,809	1,158	1,000	1,000	1,000

Table 6. Combined comparison matrix (TMP3 subcriteria).

CRI	TMP3.1			TMP3.2			TMP3.3		
TMP3.1	1,000	1,000	1,000	0,223	0,333	0,565	2,646	5,196	6,708
TMP3.2	1,769	3,000	4,486	1,000	1,000	1,000	0,939	1,316	1,592
TMP3.3	0,149	0,192	0,378	0,628	0,760	1,065	1,000	1,000	1,000

Table 7. Combined comparison matrix (TMP4 subcriteria).

CRI	TMP1.1			TMP1.2			TMP1.3			TMP1.4			TMP1.5		
TMP4.1	1,000	1,000	1,000	0,577	0,669	0,809	1,236	1,495	1,732	1,236	1,592	1,884	1,627	2,381	3,065
TMP4.2	1,236	1,495	1,732	1,000	1,000	1,000	1,236	1,592	1,884	1,236	1,495	1,732	3,482	5,904	7,297
TMP4.3	0,577	0,669	0,809	0,531	0,628	0,809	1,000	1,000	1,000	2,432	3,708	4,486	1,236	2,096	2,817
TMP4.4	0,531	0,628	0,809	0,577	0,669	0,809	0,223	0,270	0,411	1,000	1,000	1,000	0,827	1,210	1,884
TMP4.5	0,326	0,420	0,615	0,137	0,169	0,287	0,355	0,477	0,809	0,531	0,827	1,210	1,000	1,000	1,000

Table 8. Geometric means of fuzzy comparison values (Main criteria).

CRITERIA			
TMP1	2,165	2,862	3,442
TMP2	0,718	0,872	1,065
TMP3	0,210	0,252	0,354
TMP4	1,167	1,592	2,018
Total	4,260	5,578	6,879
P (-1)	0,235	0,179	0,145
INCR	0,145	0,179	0,235

Table 9. Geometric means of fuzzy comparison values (TMP1 subcriteria).

CRITERIA			
TMP1.1	3,070	4,429	5,244
TMP1.2	0,841	1,179	1,585
TMP1.3	0,244	0,300	0,440
TMP1.4	0,412	0,544	0,744
TMP1.5	0,889	1,175	1,598
Total	5,454	7,627	9,611
P (-1)	0,183	0,131	0,104
INCR	0,104	0,131	0,183

Table 10. Geometric means of fuzzy comparison values (TMP2 subcriteria).

CRITERIA			
TMP2.1	0,693	0,815	1,072
TMP2.2	0,660	0,850	1,021
TMP2.3	1,152	1,442	1,732
Total	2,505	3,108	3,826
P (-1)	0,399	0,322	0,261
INCR	0,261	0,322	0,399

Table 11. Geometric means of fuzzy comparison values (TMP3 subcriteria).

CRITERIA			
TMP3.1	0,839	1,201	1,559
TMP3.2	1,184	1,581	1,926
TMP3.3	0,454	0,527	0,738
Total	2,477	3,308	4,224
P (-1)	0,404	0,302	0,237
INCR	0,237	0,302	0,404

Table 12. Geometric means of fuzzy comparison values (TMP4 subcriteria).

CRITERIA			
TMP4.1	1,075	1,305	1,519
TMP4.2	1,457	1,839	2,104
TMP4.3	0,984	1,267	1,526
TMP4.4	0,563	0,672	0,873
TMP4.5	0,385	0,489	0,704
Total	4,463	5,572	6,726
P (-1)	0,224	0,179	0,149
INCR	0,149	0,179	0,224

Table 13. Relative fuzzy weight of each criteria.

CRITERIA				
TMP1		0,315	0,513	0,808
	TMP1.1	0,319	0,581	0,961
	TMP1.2	0,088	0,155	0,291
	TMP1.3	0,025	0,039	0,081
	TMP1.4	0,043	0,071	0,136
	TMP1.5	0,092	0,154	0,293
TMP2		0,104	0,156	0,250
	TMP2.1	0,181	0,262	0,428
	TMP2.2	0,173	0,274	0,408
	TMP2.3	0,301	0,464	0,691
TMP3		0,031	0,045	0,083
	TMP3.1	0,199	0,363	0,629
	TMP3.2	0,280	0,478	0,777
	TMP3.3	0,108	0,159	0,298
TMP4		0,170	0,285	0,474
	TMP4.1	0,160	0,234	0,340
	TMP4.2	0,217	0,330	0,471
	TMP4.3	0,146	0,227	0,342
	TMP4.4	0,084	0,121	0,196
	TMP4.5	0,057	0,088	0,158

Table 14. Averaged and normalized relative weight of criteria.

CRITERIA			Rank
TMP1	0,545	**0,506**	**1**
TMP2	0,170	**0,158**	**3**
TMP3	0,053	**0,049**	**4**
TMP4	0,310	**0,287**	**2**
TOTAL	0,481		

Table 15. Averaged and normalized relative weight of sub-criteria.

CRITERIA			
TMP1	TMP1.1	0,621	**0,559**
	TMP1.2	0,178	**0,160**
	TMP1.3	0,048	**0,044**
	TMP1.4	0,084	**0,075**
	TMP1.5	0,180	**0,162**
	TOTAL	1,110	
TMP2	TMP2.1	0,291	**0,274**
	TMP2.2	0,285	**0,268**
	TMP2.3	0,485	**0,458**
	TOTAL	1,061	
TMP3	TMP3.1	0,397	**0,362**
	TMP3.2	0,512	**0,467**
	TMP3.3	0,188	**0,172**
	TOTAL	1,097	
TMP4	TMP4.1	0,245	**0,232**
	TMP4.2	0,339	**0,321**
	TMP4.3	0,238	**0,226**
	TMP4.4	0,133	**0,126**
	TMP4.5	0,101	**0,095**
	TOTAL	1,057	

Table 16. Ranking of criteria and subcriteria according to their global weight.

Criteria and Sub-criteria	Global weights	Criteria ranking	Sub-criteria ranking
TMP1	0,506	1	
TMP1.1	0,283		1
TMP1.2	0,081		4
TMP1.3	0,022		14
TMP1.4	0,038		10
TMP1.5	0,082		3
TMP2	0,158	3	
TMP2.1	0,043		8
TMP2.2	0,042		9
TMP2.3	0,072		5
TMP3	0,049	4	
TMP3.1	0,018		15
TMP3.2	0,023		13
TMP3.3	0,008		16
TMP4	0,287	2	
TMP4.1	0,067		6
TMP4.2	0,092		2
TMP4.3	0,065		7
TMP4.4	0,036		11
TMP4.5	0,027		12

CONCLUSION

Tractors have a very important place in the agricultural mechanization system. Tractors, which constitute the basic power source for all tools and machines used in agriculture, are one of the indispensable tools of modern agriculture today. With the rapid change in technology, there has also been a rapid development in tractors. In order to increase the performance of agricultural machinery, especially tractors, choosing the right maintenance planning has become a necessity in developing and developed countries.

Due to deficiencies in the maintenance planning of agricultural machines used in agricultural activities, more expenses are required for maintenance and repair, especially for tractors. This negatively affects crop productivity in agriculture. To develop an optimal preventive tractor maintenance planning (TMP) for this purpose; It is very important in reducing maintenance costs and increasing machine performance. Fuzzy logic and multi-criteria decision making (MCDM) are useful for tractor maintenance planning (TMP) due to the nature of the agricultural sector, where there are many uncertainties.

For this purpose, choosing the right MCDM approach with fuzzy methods in an environment of uncertainty due to the nature of the agricultural sector; It has an important role in evaluating the problem according to a set of criteria. The study offers a different perspective to farmers and other stakeholders by using the fuzzy approach for tractor maintenance planning in the agricultural sector. On the basis of the prioritization of criteria of tractor maintenance planning (TMP), it was found from the ranking that checking for all fluid levels (TMP1) ranked first. This respectively is followed by checking for general conditions (TMP4), checking for tires and wheels (TMP2) and checking for batteries (TMP3). With the tractor maintenance planning (TMP) proposed in this study, the results of this study can have a positive impact on farmers to carry out their agricultural activities using tractors that operate smoothly. In addition to the experts and practitioners involved in this study, future research may also include farmers as decision makers to improve results, as various stakeholders may reveal different preferences. From a practical perspective; The application of a combined approach that integrates expert opinion and fuzzy multi-criteria decision making is a promising approach to overcome the problem of care planning characterized by multi-criteria, multi-stakeholders and uncertainty.

This research contributes to the literature in the following ways: It is the first study in which the maintenance planning of agricultural machinery is applied with fuzzy AHP for agricultural productivity in the agricultural sector. However, there are modeling studies that examine the maintenance planning selection problem on a sector-by-sector basis using classical MCDM methods. Fuzzy AHP method, which provides ease of application, was preferred in determining the Criterion Weights. The research proposes a framework to determine the weights of appropriate criteria for care planning selection through a combined approach of fuzzy multi-criteria decision making involving relevant stakeholders.

The results and analyzes of this study are based on literature that met our selection criteria. Some limitations should be noted. For example, we based our conceptual model on tractor maintenance planning and did not include other agricultural machines. Future studies should expand the number of studies on maintenance planning to other agricultural machinery. Research on different aspects of the effects of the efficiency of farm machinery on agricultural productivity is still emerging. Future research could also examine impacts on agricultural production and agricultural productivity, and possible changes in sustainable development and economic growth.

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RESEARCH ARTICLE

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Child feeding practices and male involvement in child feeding among smallholder farming households in Uganda

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Abstract
Smallholder farmers continue to face food and nutrition insecurity. The timing, scope and effectiveness of interventions is influenced by decision-making and roles within households and communities, yet emphasis has majorly been placed on mothers. A cross-sectional study explored the child feeding practices and extent of the father's participation in child feeding among 433 farming households with children aged 6-35 months from two rural and two peri-urban districts in Central Uganda. Diets consumed by children were inadequate with only 37% meeting the minimum dietary diversity. About 65% of fathers participated in decision-making over child feeding with 10% all the time and 24% only a few times. Additionally, 48% of fathers participated in actual child feeding, 2% all the time and 23% only a few times. Main reasons for the level of father participation included the presence or absence of money or food; concern or lack of concern over the child's health; father's physical availability; and whether child feeding is part of a father's responsibility or not. Fathers who participated in decision-making regarding child feeding were more likely to participate in actual child feeding ($r=0.6$, $P<0.01$). There is an opportunity for active engagement of fathers in nutrition-related interventions to enhance their participation and support in decisions concerning child feeding and actual child feeding for enhanced child and household food and nutrition security.
Keywords: Gender, Father, Child nutrition, Dietary diversity, Decision-making

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INTRODUCTION

Smallholder farmers produce majority of the food in developing countries yet remain vulnerable to poverty, climate change, food insecurity, hunger, and malnutrition, especially undernutrition and micronutrient deficiencies (van Wijk et al., 2018; Williams et al., 2018). To address these challenges, approaches have included the promotion of sustainable production, increase of incomes from agriculture through markets, diversification of production beyond starchy staples, promotion of nutrition within agricultural initiatives, empowerment of women and utilization of multi-sectoral approaches (FAO et al., 2018). The impact of these approaches is, however affected by the decision-making and power relations within households and communities. To understand and harness decision-making and power relations approaches such as the Gender Action Learning System and Nurturing Connections have been developed. These approaches explore the gender and intra-family dynamics that influence production, food security, nutrition and health (HKI, 2015; PELUM, 2016).

When it comes to child nutrition and care practices, most emphasis has been placed on the first 1000 days of a child's life thus narrowing the focus of

interventions on children and mothers as the primary caregivers and target beneficiaries within households. Studies have reported the relationship between mother's knowledge and child feeding practices and nutrition status (El-Nmer et al., 2014; Fadare et al., 2019; Saaka, 2014). As a result, efforts have been made to enhance the knowledge and skills of mothers (Saaka et al., 2021; Sanghvi et al., 2013; Waswa et al., 2015). Beyond the mother as a caregiver, the influence and participation of grandmothers in child-feeding practices has also been noted, highlighting the need for their involvement and ensuring adequate information in a community (Aubel, 2012; Nankumbi & Muliira, 2015).

The roles and responsibilities of fathers in child feeding and the kind of feeding practices fathers employ have been reported in developed countries (Khandpur et al., 2014). The association between fathers' education level and nutrition status of children in developing countries has been reported and the role of fathers in child nutrition is increasingly coming under focus (Bilal, 2015; Kansiime et al., 2017; Kikafunda & Tumwine, 2007; Willey et al., 2009). There is, however, limited documentation of the extent of involvement of fathers in child feeding in developing countries. If the effectiveness and impact of nutrition and nutrition-sensitive agricultural interventions is to be increased, a further look at the existing household dynamics and social support is needed. Nutrition remains important given the continued gaps. Nationally, 29% of children aged 6-59 months in Uganda are stunted and 53% are anemic (UBOS & ICF, 2018). In addition, 30% of children aged 6-23 months consume diets with minimum dietary diversity, 42% have minimum meal frequency and only 15% have the minimum acceptable diets (UBOS & ICF, 2018).

This study therefore explored the feeding practices of children aged 6-35 months and role of fathers in child feeding within farming households in rural and peri-urban districts of Uganda. It was conducted as part of a larger program, the CGIAR Consortium of International Agricultural Research Centers research program on integrated systems for the humid tropics. The HumidTropics program research focused on improving livelihoods and productivity of poor smallholder farming communities based on the dominant integrated systems they depend on in tropical Africa, Asia, and Americas. In East and Central Africa, the program generated agricultural innovations for improved livelihoods for smallholder farmers using multi-stakeholder processes and partnerships (CGIAR Research Program on HumidTropics, 2016).

MATERIALS AND METHODS

A cross-sectional mixed methods survey using a semi-structured questionnaire was conducted in four districts in Central Uganda, that is Kiboga, Kyankwanzi, Mukono, and Wakiso districts. The four districts were clustered to form two groups based on the level of urbanization. Kiboga and Kyankwanzi districts are agriculture-dependent districts located in the north-central part of the country and are largely rural districts with 77% and 89% residing in rural areas respectively (UBOS, 2016). Mukono and Wakiso districts on the other hand are located in the south-central part of the country and are largely peri-urban with population densities above the national average of 177persons/km^2 (Ekesa et al., 2015). Mukono and Wakiso districts have 73% and 41% of their populations residing in rural areas respectively (UBOS, 2016). Even with more off-farm employment or sources of income in Mukono and Wakiso, there is still significant ongoing agricultural production (Ekesa et al., 2015).

A multi-stage systematic random sampling procedure was used. Districts are made up of sub-counties, parishes, and villages. The districts, sub-counties and parishes were purposively selected based on where the HumidTropics program was implemented. A total of fifteen villages were randomly selected in each district, from which households were randomly sampled. A list of households meeting the study criteria in each village was created with the help of community leaders from which a list of random numbers was generated using Microsoft Excel to sample households. The inclusion criteria included farming households with at least one child aged 6-35 months. For households with multiple children in the specified age range, the youngest child was selected for the study. The total sample size of 433 households was calculated based on formulars by Magnani where the proportion of households with children aged 6-35 months in the sampled Parishes were used (Magnani, 1999). The proportions were as follows, Kiboga 8%, Kyankwanzi 6%, Mukono 7%, and Wakiso 7% (unpublished reports). The survey was conducted in November-December 2016 within the national research mandate of HumidTropics program implementing partners and the National Agricultural Research Organization. For this study, local approval was obtained from the respective district authorities. Informed consent was obtained from the respondents prior to the interview. The questionnaire was pre-tested prior to data collection. The survey was conducted by trained enumerators at the respective households.

Data collection

The survey used a semi-structured questionnaire that collected information from the household head or spouse on household characteristics, child food consumption, and participation of fathers in child's feeding. A qualitative 24-hour recall was used to obtain the number of meals and type foods consumed by the children Using open recall, respondents were asked to describe all the food and drinks that the child consumed throughout the day, probing for details of mixed/composite dishes. Minimum recommended dietary diversity for children aged 6-23 months

was assessed using seven food groups where consumption of a food group received a score of one. Children with total scores ≥4 were considered to have met the minimum dietary diversity requirement of four food groups. The food groups were (i) cereals, white roots, tubers, bananas (ii) legumes (iii) milk and milk products, (iv) eggs, (v) meat and fish, (vi) vitamin A-rich fruits and vegetables, (vii) other fruits and vegetables (WHO et al., 2010). The minimum meal frequency was also determined based on World Health Organization guidelines where breastfed children are recommended to have two meals at six to eight months and three meals at nine to 23 months and non-breastfed children four meals a day. The minimum acceptable diet was then established when a child met the minimum dietary diversity and meal frequency (WHO et al., 2010). The questionnaire also captured child age, breastfeeding practices, and access to information on child feeding.

The extent of the fathers' participation in a) decision-making regarding the feeding of the child and b) taking part in the feeding was rated by the respondents using a 5-point scale as follows: Not at all 0% of the time; A few times <25% of the time; Half of the time >25-50% of the time; Most of the time >50% of the time; or All the time 100%. While largely quantitative, the data collection tool also included open-ended questions to capture the reasons for the level of participation reported. The reasons were noted in full, and probing was conducted where necessary to ensure the situation regarding the father and household was well understood and recorded by the enumerator.

Data analysis

SPSS version 23 was used to summarize data using descriptive statistics, conduct cross tabulations and chi square tests to compare variables in Kiboga-Kyankwanzi districts and those in Mukono-Wakiso districts, and conduct bivariate correlations using Spearman correlation analysis to establish any relationships between participation of fathers and other child feeding variables. Results for Kiboga and Kyankwanzi districts are clustered together as the rural districts, while Mukono and Wakiso districts are clustered together as the peri-urban districts.

The reasons for the respective levels of participation from the open-ended questions were analyzed in Microsoft excel by identifying themes and codes across responses, coding responses, and grouping of codes into a matrix. The matrix was used to understand how the themes and codes interact. In addition, the frequency of the codes and themes across districts was also generated.

RESULTS

Household characteristics

Of the 433 households surveyed, 376 households (87%) had complete data on child feeding practices and participation of fathers and were therefore included in the analysis. Half of the households were from Kiboga-Kyankwanzi districts and the other half from Mukono-Wakiso districts (28% Kiboga, 22% Kyankwanzi, 25% Mukono and 26% Wakiso). The average respondent age in years was 29.2±8.8, and household size was 6.1±2.8 members. Sixteen percent of households were headed by women (table 1). Majority of respondents were monogamously married (60%), 17% were single, 12% polygamously married, and 10% separated, divorced, or widowed. Arable farming and mixed farming were the primary source of income for 57% and 27% of households in Kiboga-Kyankwanzi districts respectively, while in Mukono-Wakiso districts, business/trade and arable farming were the primary source of income for 36% and 25% of households, respectively. Men and women were each reported to be separately involved in the primary income activity by 35% of the households, while joint participation of men and women was reported in 24% of households. Decision-making over the income from the primary source was made by men in 43% of households, by women in 32% and by both men and women in 20% of households.

Table 1. Household demographics and characteristics

Characteristic		Kiboga-Kyankwanzi (n=188, Rural)	Mukono-Wakiso (n=188, Peri-urban)	Total (n=376)	Chi-square values
Respondents	Women	89.4	96.3	92.8	6.670**
	Men	10.6	3.7	7.2	
Household head	Women	16.0	16.5	16.3	2.208
	Men	84.0	83.5	83.7	
Age of respondent	≤25	41.0	38.5	39.7	1.633
	26-40	50.0	53.5	51.7	
	41-55	8.0	5.9	6.9	
	>55	1.0	2.1	1.6	

Characteristic		Kiboga-Kyankwanzi (n=188, Rural)	Mukono-Wakiso (n=188, Peri-urban)	Total (n=376)	Chi-square values
Marital status of respondent	Single	16.5	17.7	17.1	2.246
	Monogamously married	60.1	59.7	59.9	
	Polygamously married	13.3	11.3	12.3	
	Widowed	0.5	2.2	1.3	
	Separated/Divorced	9.6	9.1	9.4	
Education level of most educated household member	No formal	5.4	0.5	3.0	44.851***
	Primary	34.6	26.2	30.4	
	Secondary	45.9	56.3	51.1	
	Post-secondary	14.1	16.9	15.5	
Primary source of income	Arable farming [a]	57.4	24.6	41.1	131.277***
	Cattle farming	0.5	8.0	4.3	
	Mixed farming [a]	27.1	4.3	15.7	
	Casual labor	1.1	8.0	4.5	
	Employed	4.8	17.6	11.2	
	Business/trade	9.0	35.9	22.4	
Participation in primary source of income	Women	37.3	31.7	34.5	28.300***
	Men	25.9	44.6	35.3	
	Joint (both men & women)	33.0	15.1	24.0	
	Children	1.6	3.2	2.4	
	Elders	2.2	5.4	3.8	
Decision-making regarding income from primary source of income	Women	29.4	33.2	31.9	6.352
	Men	41.7	41.7	42.5	
	Joint (both men & women)	21.9	18.2	20.4	
	Children	1.1	2.7	1.9	
	Elders	2.1	4.3	3.3	
Average number of meals per day in household	One	8.1	3.8	5.9	13.802***
	Two	37.1	23.1	30.1	
	Three or more	54.8	73.1	64	

Figures are percentages of households. [a] Arable farming: engaging in crop production alone. [b] Mixed farming: production of both crops and livestock. Pearson Chi-square values: ***significant at 1%, **significant at 5%, *significant at 10%.

Child health and feeding practices

The distribution of children by age showed that 26% were 6-11 months, 53% were 12-23 months old and 21% were 24-35 months old. The rate of exclusive breastfeeding, that is, provision of only breastmilk up to six months of age was at 77% (table 2). Cow's milk and maize porridge (a liquid porridge) were the most common substitutes for breastmilk that were given before children were 6 months old (results are not presented in the table). For children who were not exclusively breastfed, 52% and 41% in Kiboga-Kyankwanzi were fed cow's milk and porridge respectively, and in Mukono-Wakiso, consumption 55% and 23% respectively. Half of the children aged 6-23 months were still breastfeeding at the time of the survey (table 2).

The children's diets were mainly composed of starchy staples and legumes that were consumed by 92% and 65%, respectively (table 2). Milk consumption was at 44% while meat and fish were consumed by only 24%. Less than half of the children consumed fruits and vegetables in the preceding 24 hours, with 30% having consumed vitamin A rich fruits and vegetables. More legume consumption was noted in Kiboga-Kyankwanzi while more milk and other fruits and vegetables were consumed in Mukono-Wakiso. Overall, only 37% of the children aged 6-23 months met the minimum dietary diversity requirement of four food groups.

Among children aged 6-23 months, 36% met the minimum dietary diversity while 34% had the minimum recommended meal frequency. As a result, only 16% of children aged 6-23 months consumed the minimum acceptable diets. The proportion of children meeting the minimum acceptable diets was higher in Mukono-Wakiso compared to Kiboga-Kyankwanzi (22% compared to 10%) (table 2).

Fifty six percent of households reported to have received information on child feeding at least once in the past six months. This information was mainly obtained from health centers (39%), community health workers (25%) and radios (23%) (table 2).

Table 2. Child feeding practices

Variable		Kiboga-Kyankwanzi (n=188, Rural)	Mukono-Wakiso (n=188, Peri-urban)	Total (n=376)	Chi-square values
Sex	Girls	51.6	54.0	52.8	0.219
	Boys	48.4	46.0	47.2	
Age (months)	6-12	27.1	24.6	25.9	1.796
	13-24	50.0	56.7	53.3	
	25-35	22.9	18.7	20.8	
Exclusively breastfed		83.0	70.8	76.9	7.782***
Currently breastfeeding	6-23 months (n=297)	61.4	50.3	55.7	3.104*
	≥24 months (n=79)	20.7	5.6	14.9	
Dietary diversity	Low DDS	70.2	55.6	62.9	8.564***
	Minimum DDS	29.8	44.4	37.1	
Food group consumption	Starchy staples	92.0	92.0	92.0	0.000
	Legumes	78.2	51.3	64.8	29.641***
	Milk	37.8	50.8	44.3	6.458**
	Eggs	11.2	7.0	9.1	2.023
	Meat and fish	19.1	27.8	23.5	3.913*
	Vitamin A rich fruits and vegetables	31.4	28.3	29.9	0.414
	Other fruits and vegetables	36.2	60.4	48.3	22.093***
Diets of 6-23-month-old children	Minimum DDS	28.9	42.3	35.8	6.318**
	Minimum meal frequency	25.5	41.4	33.7	8.432***
	Minimum acceptable diets	9.7	22.4	16.2	8.852***
Received information on child feeding		50.3	61.6	55.9	4.864**
Source of information on child feeding	Community health worker	34.8	16.7	24.6	37.710***
	Health center	21.3	52.6	38.9	
	Radio	21.3	23.7	22.7	
	Mother/mother-in-law	0.0	0.9	0.5	
	Television	0.0	0.9	11.8	
	NGO	22.5	5.3	12.8	

DDS: Dietary Diversity Score. NGO: Non-Governmental Organization. Figures are percentages of households. Pearson Chi-square values: ***significant at 1%, **significant at 5%, *significant at 10%

Participation of fathers

According to the respondents, more than half of the fathers (65%) participated in decision-making over child feeding. Of these 10% participated all the time and 24% participated a few times (figure 1). Participation of fathers in child feeding was lower compared to participation in decision-making. Less than half of the fathers (48%) participated in child feeding with only 2% participating all the time, 22% half/most of the time, and 23% participating a few times. The reasons given for the level of participation centered around presence or absence of money or food, concern, or lack thereof on the child's health, physically availability of the father in the household, child feeding being part of the father's responsibility or not, and other household dynamics (table 3).

The specific reasons for the fathers' participation in decision-making over child feeding were having an income (31%), desire for healthy children (21%), and physically unavailability of the father (14%) (table 4). It was also reported by 8% that participation in decision-making over child feeding was the responsibility of the father while for 9%, it was not considered as their responsibility. The availability or lack of money was a major factor influencing participation of 38%, 42% and 38% of fathers that participated few times, half/most of the time, and all the time, respectively. This was followed by the desire for healthy children, a reason given for 19%, 31% and 35% of fathers that participated a few

times, half/most of the time, and all the time respectively. For those that did not participate in decision-making at all, it was mainly attributed to the physical unavailability of the father (39%) and perception that child feeding was not the fathers' responsibility (28%). The availability of income as a factor was higher in Mukono-Wakiso (34%) compared to Kiboga-Kyankwanzi (26%). And so was the physically unavailability of the father at 17% in Mukono-Wakiso compared to 10% in Kiboga-Kyankwanzi. On the other hand, desire for healthy children and child feeding being part of the father's responsibility was higher in Kiboga-Kyankwanzi (29% and 13%, respectively) compared to Mukono-Wakiso (15% and 5%, respectively).

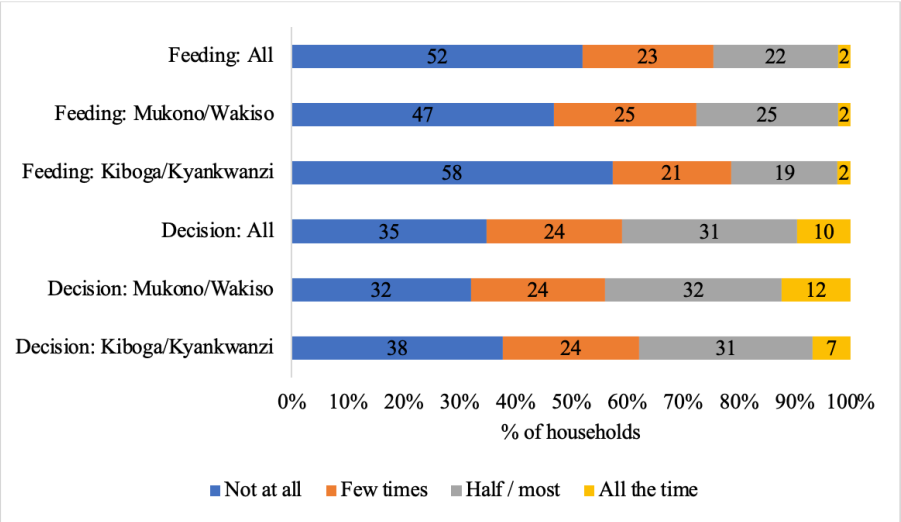

Figure 1. Percentage of participation of fathers in decision-making and child feeding

Decision: decision-making over child feeding; Feeding: participation in child feeding; Half/ most of the time: includes fathers that particpated half of the time and most of the time

Table 3. Definition of the reasons for the level of participation of fathers in decision-making and child feeding

Theme of reasons	Reasons contributing to participation	Reasons inhibiting participation
1. Money/income	There is money in the household He buys food He controls the income	There is no money He does not buy food
2. Food availability	He harvests food from the field He brings food home He keeps part of the harvest for household food He keeps food for children	Food is not enough/ there is no food
3. Healthy children	He wants healthy children He knows what to feed the children He ensures children are growing well	The mother knows best
4. Physical availability	He is around/ always at home	He is never around He works or stays far Child is living with the mother (away from the father)
5. Responsibility	He loves and cares for the children Children are his responsibility He is a good father and is actively involved He inquires/questions their feeding	He has no time He is not interested in the children/ their feeding He does not take care of the family
6. Household dynamics	Grandfather wants children to grow well Mother is pregnant so he takes over child feeding	Parents are separated or divorced He has another family

Definitions are a summary of the responses as given by respondents; He refers to the father

Table 4. Distribution of the major reasons given for the level of participation of fathers in decision-making and child feeding

	Reason	Kiboga-Kyankwanzi (n=188, Rural)	Mukono-Wakiso (n=188, Peri-urban)	Total (n=376)
Decision-making	Availability of money	25.6	34.3	30.5
	Interest in the child's health	28.6	15.4	21.1
	Father not physically available	10.5	17.1	14.3
	Not the fathers' responsibility	7.5	10.3	9.1
	Part of the fathers' responsibility	12.8	4.6	8.1
	Negative household dynamics	6.0	9.7	8.1
	Other reasons	9.1	8.5	8.7
Child feeding	Not the fathers' responsibility	25.2	25.9	25.6
	Part of the fathers' responsibility	15.1	21.0	18.5
	Father not physically available	11.8	16.7	14.6
	Interested in the child's health	19.3	5.6	11.4
	Negative household dynamics	5.9	13.0	10.0
	Father is physically available	10.9	7.4	8.9
	Other reasons	11.7	10.4	11.1

Figures are percentages of households

The main reasons for the level of participation of fathers in actual child feeding included child feeding not being perceived as part of the fathers' responsibility (26%), child feeding being part of the fathers' responsibility 19% and the physical unavailability of the father 15% (table 4). The responsibility of the father was a factor for those that fed the child a few times (24%), half/most of the times (35%), and all the time (43%). Desire for healthy children was the second highest ranked factor and was the reason behind fathers who fed their child half/most of the time (27%) and all of the time (29%). For those that did not participate in feeding at all, it was attributed to it not being their responsibility (46%), their physical unavailability (24%), and other household dynamics (23%). Child feeding being part of the responsibility of the father and the physical unavailability of the father were reported more in Mukono-Wakiso (21% and 17%) compared to Kiboga-Kyankwanzi (15% and 12%). On the other hand, desire for healthy children was higher in Kiboga-Kyankwanzi 19% compared to Mukono-Wakiso 6%.

Participation of fathers in decision-making was significantly associated with participation in child feeding ($r=0.6$, $P<0.01$), indicating that fathers that engage in decision regarding child feeding were more likely to participate in feeding the child. There was a weak and significant positive association between dietary diversity of children and the father participating in decision-making ($r=0.2$, $P<0.01$) and child feeding ($r=0.2$, $P<0.01$). Weak but significant negative associations were found between participation of fathers in decision-making with receiving information on nutrition ($r=-0.1$, $P<0.01$).

DISCUSSION

Sixty five percent of fathers participated in decision-making over child feeding, with 10% participating all the time and 24% only a few times. In addition, only 48% of fathers participated in child feeding with only 2% participating all the time and 23% only a few times. The main reasons for these levels of participation were availability of income, desire for healthy children, child feeding being part of the fathers' responsibility or not, and the physical unavailability of the father. The main reasons for the father's participation varied with the districts. Compared to the rural districts (Kiboga-Kyankwanzi), the peri-urban districts (Mukono-Wakiso) had less children with low dietary diversity and more fathers participating in child feeding.

The gaps in the quality of child feeding practices were similar to those reported elsewhere. The low dietary diversity, low consumption of the minimum recommended acceptable diets, and low consumption of fruits and vegetables observed were similar to the national reports (UBOS & ICF, 2018). The consumption patterns and gaps in diet quality in the rural districts (Kiboga-Kyankwanzi) compared to the peri-urban districts (Mukono-Wakiso) were not expected, because despite the peri-urban districts having higher urbanization and lower estimated poverty levels (UBOS, 2019), more than half of the children had low dietary diversity. This could be attributed to the significant proportion of rural households/ subsistence farming households in both rural and peri-urban areas and low income levels in the peri-urban districts (UBOS, 2016). It is also likely that there is a lack of nutrition knowledge coupled with feeding patterns that have not improved even as the status of the communities are changing with urbanization. This highlights the

need for studies on the changing food environment in peri-urban areas and corresponding interventions to enhance the food and nutrition security.

The observed levels of participation of fathers in child feeding were corresponded to previous reports in Southwest Uganda where 52% of fathers participated in child feeding and 23% made decisions on complementary feeding. Other major roles of fathers reported that are in tandem with this study include participation in farming, providing money, and providing information about child feeding (Kansiime et al., 2017). A study in Ethiopia where child dietary diversity was also low, reported an association between involvement of fathers in child-care and feeding with dietary diversity particularly in urban areas (Bilal et al., 2016). Child care and feeding were perceived to be the role of the mother though a change in perceptions and an increase in involvement of fathers was noted (Bilal et al., 2016). From the reasons supporting the participation of fathers in decision-making and child feeding, the study revealed several perceptions about the importance of child feeding and the roles and responsibilities of fathers. While there were households that relegated the importance and responsibility of child feeding to mother, there were households that also considered child feeding apart of the responsibility of the father.

Involvement of fathers in child feeding and modelling of healthy behaviors has been associated with lower child nutrition risk while their education status has been associated with the nutrition status and feeding practices of children (El-Nmer et al., 2014; Jesmin et al., 2011; Watterworth et al., 2017). Indeed, participation of fathers and adequate child feeding practices require having nutritional knowledge and skills that support quality diets. Though our study did not explore the father's knowledge, parents having adequate nutritional knowledge has been related to the child's dietary intake particularly dietary diversity (El-Nmer et al., 2014), and child feeding practices have improved following nutrition education (Ickes et al., 2017; Kajjura et al., 2019). On the other hand, there appear to be different dynamics between the father's education level versus that of the mother. A study in western Uganda noted that the father's education level did not independently predict any child feeding practice unlike that of the mothers (Wamani et al., 2005). This could be related to mothers being the primary caregivers and the norms and perceptions around the roles and responsibilities of fathers.

Nutrition education and awareness creation campaigns as well as food and nutrition security interventions should therefore also actively target and involve fathers. For example by having behavior change and communication strategies that include fathers and ensuring that implementors such as community health extension workers actively engage fathers during implementation activities (Bilal, 2015). In addition, the perceptions about the importance of child feeding and the roles and responsibilities of fathers from this study provide insight on the motivators and barriers, which can be used to design the behavior change communication strategy and messages (Skinner, 2019). For example, the interest in and pride that comes with having healthy children can be used to attract fathers to messages about child feeding and nutrition; role models can be used to showcase the roles and responsibilities of fathers; and messaging that addresses the physical unavailability of some fathers could be developed. Following a study of dietary patterns of fathers, Ochieng et al. (2017) recommended a reduction in the food consumed away from home by fathers and utilization of savings towards nutritious foods for the household during times of food shortages (Ochieng et al., 2017). Such approaches could be included in the behavior change and communication strategies. Indeed interventions that utilize whole household approaches, equipping the mother, father and other key household and community members have the potential to increase impact (Ekesa et al., 2018).

Household information on nutrition was mainly from health centers, community health workers and radios. This underscores the need to maximize the different avenues through which information is disseminated in both rural and peri-urban areas. Whereby regardless of the avenue (health centers, community health workers, radios, mobile phones, television, etc.), the messaging targets both mothers and fathers.

CONCLUSION

Moderate to low participation of fathers in child feeding was noted with more fathers participating in decision-making compared to child feeding. Income, responsibilities of the father, and desire for healthy children were some of the key reasons influencing the level of participation of fathers. There is an opportunity for active engagement of fathers in nutrition and nutrition-related interventions to build their capacity and thus enhance their participation in household decision-making and child feeding. An increase in awareness, knowledge and skills through behavior change and communication strategies that target both mothers and fathers and strategies that utilize the motivators and barriers identified in this study could potentially increase the participation of fathers and contribute towards enhanced household food and nutrition security and child nutrition outcomes.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Isorhamnetin as a promising natural bioactive flavonoid: *in vitro* assessment of its antifungal property

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Abstract

Isorhamnetin (3'-methylquercetin) is an important flavonoid produced as a secondary metabolite from medicinal and aromatic plants due to its pharmacological and therapeutic properties. Thus far, the anticancer, antiallergic, antiinflammatory, antioxidant, antiviral, and antimicrobial properties of isorhamnetin have been evaluated in indirect studies conducted with isorhamnetin found in plant extracts or essential oils or direct studies performed with pure isorhamnetin. However, this is the first study in the literature on the antifungal activity of 97% pure isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and C. *parapsilosis* using two assays including agar dilution and broth microdilution methods. This study showed that isorhamnetin has a significant inhibitory effect against all *Candida* species used. The minimum inhibitory concentration (MIC) value of isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis* was 1.875 mg/mL, the same for all yeast strains. These results have opened a new horizon regarding the usability of isorhamnetin as a pharmacological therapeutic antifungal agent.

Keywords: Isorhamnetin, Flavonoids, Antifungal Activity, Pharmaceutical compound, Therapy

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Introduction

The severity of fungal diseases varies depending on the type of yeast and the area of the body infected. *Candida* species affects the cutaneous layer and mucous membrane and causes infections in the mouth, skin, vagina, and intestines (Talapko et al., 2021; Odds, 1994). If the host immune system is not functioning properly, *Candida* infection can spread to other surrounding areas of the heart or brain, causing severe symptoms and invasive candidiasis (Kuran, 2021; Pappas et al., 2018). If it is not controlled, *Candida* penetrates the bloodstream by breaking down the intestinal walls in the digestive system. It can cause leaky gut syndrome by releasing the toxins it synthesizes (Kuran, 2021). The proportion of non-*albicans Candida* species among *Candida* species that cause fungal diseases is increasing yearly and constitutes 35-65% of all fungal diseases. (Krcmery and Barnes, 2002). The most common non-*albicans Candida* species are *C. parapsilosis*, with a rate of 20-40%; *C. tropicalis*, with a rate of 10-30%; and *C. krusei*, with a rate of 10-35%. While the mortality rate due to *C. albicans* is around 20-40%, the mortality rate due to non-*albicans Candida* species is between 15% and 35% (Krcmery & Barnes, 2002).

A study reported that more than $7.2 billion was spent on treating fungal diseases in the United States between 2005 and 2014, and *Candida* infections constitute the most crucial part of fungal diseases (Benedict et al., 2019). In Turkiye, the frequency of fungal infections in hospitalized patients has increased

significantly in the last 20 years (Karakoç, 2019). Fungal infection was detected in 12.5% of hospitalized patients, including intensive care units. 1/3 of fungal infections were isolated from the intensive care unit (Özçetin et al., 2009). The treatment process of fungal disease is carried out with intensive antibiotic and antifungal treatment. It is known that long-term antibiotic and antifungal use causes harm to the body (Benitez & Carver, 2019). The antibiotics used may cause side effects such as allergic symptoms such as itching and rashes on the skin, difficulty breathing, sudden low blood pressure, rapid heartbeat, and loss of consciousness. Digestive system disorders such as diarrhea, constipation, nausea, vomiting, bloating, indigestion, loss of appetite, and abdominal pain are among the harmful effects of antibiotics (Ekici, 2023). These are chemical treatment agents that have therapeutic properties but are not natural. Antifungal drug resistance is a significant problem in many fungal diseases, and its incidence in medical centers has increased in recent years (Fong, 1996; Cowen et al., 2014). For all these reasons, researchers have focused on natural antifungal substances. Plant-derived natural substances used in traditional medicine have been preferred due to their easy accessibility, cheapness, and widespread availability.

In traditional medicine, plant extracts from medicinal and aromatic plants or phenolic substances from these plants have been used for centuries. Medicinal plants are used in primary health care for their antimicrobial, anticancer, antithrombotic, antidiabetic, and many disease-therapeutic properties (Gong et al., 2020; Senizza et al., 2020; Man et al., 2019; Knezevic et al., 2016). Isorhamnetin is an important compound obtained from some of these medicinal plants (Ren et al., 2019; Pengfei et al., 2009; Teng et al., 2006). Isorhamnetin (molecular formula: $C_{16}H_{12}O_7$), called 3'-methylquercetin, is a natural compound and flavonoid with high pharmaceutical value. Isorhamnetin is a monomethoxyflavonol produced as a secondary metabolite, especially from medicinal and aromatic plants (Zou et al., 2023; Gong et al., 2020). Studies have reported that Isorhamnetin has antioxidant activity in the vascular smooth muscle and atrium of experimental animals, as well as cytoprotective and cardiovascular activity (Li et al., 2022; Xu et al., 2020; Kuti, 2004; Ibarra et al., 2002). It has been proven that isorhamnetin has pharmacological effects on various cancer cells (Hu et al., 2015; Li et al., 2014; Kim et al., 2011; Jaramillo et al., 2010). Isorhamnetin obtained from the plant source has proven anticancer activity against hepatocellular carcinoma cells and has been shown to induce cell cycle arrest in the G1 phase (Teng et al., 2006). Furthermore, isorhamnetin can prevent Alzheimer's disease and has effective pharmacodynamics against hyperuricemia and pulmonary fibrosis (Ishola et al., 2019; Adachi et al., 2019; Zheng et al., 2019). There are many pharmacological studies about isorhamnetin in the literature, but this is the first study of the antifungal activity of pure isorhamnetin against *Candida* species. This study investigated the antifungal activity of 97% pure isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis* using agar dilution and broth microdilution methods.

MATERIALS AND METHODS

Isorhamnetin

The flavonoid isorhamnetin (3,5,7-Trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one) was purchased from BLDpharmatech (CAS#: 480-19-3, Shanghai, China). The molecular weight of isorhamnetin is 316.26, and 3'-methylquercetin is the synonym of isorhamnetin. The structural formulation of isorhamnetin is shown in Figure 1.

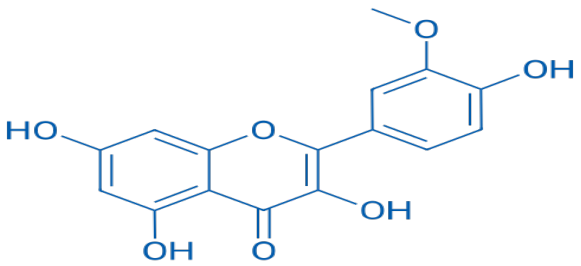

Figure 1. Chemical structure of isorhamnetin (3'-methylquercetin).

Strains and Media

The Minimum Inhibitory Concentration (MIC) values against yeast strains were determined according to the dilution methods (Unver et al., 2023). In this study, 4 yeast strains were used, including *Candida tropicalis* (ATCC 13803), *Candida albicans* (ATCC 14053), *Candida krusei* (ATCC 14243) *and Candida parapsilosis* (ATCC 22019) strains were used. All strains were purchased from the American Type Culture Collection (ATCC). They were subcultured on sabouraud 4 % glucose agar (Chemsolute, Renningen, Germany) and incubated at 35 °C for 24 hours. Subsequently, sabouraud broth (Biolife,

Milan, Italy) and sabouraud glucose agar were used for antifungal microdilution and agar dilution assays, respectively.

Antifungal Assay

Broth Dilution Assay

6 mg of isorhamnetin was dissolved in 100 µL dimethylsulfoxide (DMSO) (Honeywell, Germany), and 25 µL of each solution was added to the first well containing 175 µL of broth. The first well contained 7.5 mg/mL isorhamnetin, with a total volume of 200 µL. After that, a 100 µL sample from the first well was added to the second line of the microtiter plate and serially two-fold diluted with sabouraud broth from the 1st to the 10th well. The concentration of isorhamnetin in the wells ranged from 3.75 to 0.007 mg/mL. The standard inoculum was prepared from yeast species, and the density of each species was adjusted to 0.5 McFarland (1-1.5 × 10^6 CFU/mL). 1 µL of the prepared standard inoculums of *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis* were inoculated into A, B, C, and D rows, respectively. The wells in the 11th row were set as a positive control that did not contain isorhamnetin, proving the viability of the microorganism used. The 12th row wells were the negative control, confirming that only broth medium was used and there was no contamination. The microplate was incubated at 35 °C for 24 h. The next day, 10 µL of resazurin (0.15% w/v) was added to each well to determine the growth of *Candida* species, and the microplate was left in an incubator at 35 °C for 3-4 h. Microorganisms are observed to grow in the wells where blue turns to pink. The experiments were carried out in triplicate, with zero standard deviation.

Agar Dilution Assay

In the agar dilution method, the antifungal activity assay was repeated to confirm the MIC values of isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis.* 45 mg of isorhamnetin was dissolved in 1,5 mL DMSO, and this solution was added into 10.5 mL of sabouraud glucose agar at 50 °C. Two-fold dilution was done to obtain an isorhamnetin concentration of 22.5 mg/6 mL in the first agar plate. Therefore, the concentration of isorhamnetin in the agar plates from 1st to 11^{th} ranged from 3.75 to 0.003 mg/mL. After the agar plates were allowed to cool and solidify, they were divided into four areas, and each *Candida* strain was inoculated in these areas separately. A pure sabouraud glucose agar plate was used as a control plate. The standard inoculum of each *Candida* species was prepared in distilled water, and their turbidity was set to 0.5 McFarland. 1 µl of *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis* were inoculated from the standard inoculum onto the agar plates using a blue loop. Subsequently, all plates were incubated at 35°C for 24 h. The next day, the growth of *Candida* species on the plates was evaluated.

Results and Discussion

Results of the Broth Microdilution Method

According to the antifungal activity using the broth microdilution method, MIC values are the values in the well where there is no color change, and the lowest isorhamnetin concentration is used. In Figure 2, it can be seen that the color change begins after the third well. Therefore, the MIC value of isorhamnetin against *C. tropicalis*, *C. albicans*, *C. krusei*, and *C. parapsilosis* was found to be 1.875 mg/mL, which is the concentration in the second well, being the same against all microorganisms (Figure 2). All tests were performed in triplicate, and the standard deviation was zero.

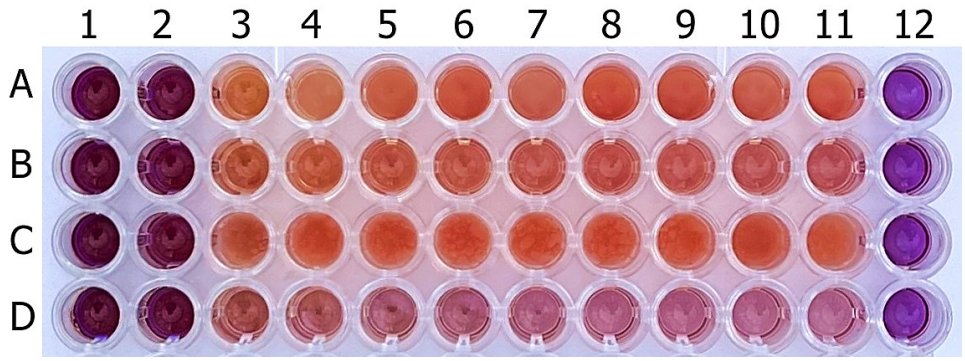

Figure 2. Microplate picture of Isorhamnetin antifungal assay against *C. tropicalis* (A), *C. albicans* (B), *C. krusei* (C), and *C. parapsilosis* (D) using sabouraud broth. 1-10 wells include different concentrations of isorhamnetin (from 3.75 to 0.007 mg/mL) 11. positive control, 12. negative control.

Results of the Agar Dilution Method

The antifungal activity results of isorhamnetin against *C. tropicalis*, *C. albicans*, *C. krusei*, and *C. parapsilosis* using agar dilution assay are illustrated in Figure 3. Plates left incubated at 35 ˚C overnight were evaluated for antifungal activity results. As a result, it was observed that all *Candida* species, including *C. tropicalis*, *C. albicans*, *C. krusei*, and *C. parapsilosis*, showed significant growth on the C plate, where the isorhamnetin concentration was 0.937 mg/mL. No colonies were detected on A and B plates treated with 3.75 and 1.875 mg/mL isorhamnetin, respectively. The MIC is defined as the lowest concentration at which the substance inhibits the microorganisms used in the study, as explained by the Clinical and Laboratory Standards Institute (CLSI, 2018). In this study, the MIC value is described as the lowest concentration of isorhamnetin at which visible growth of the microorganism on the plates is inhibited. Therefore, the concentration (1.875 mg/mL) of isorhamnetin in the B plate was determined as the MIC value for all yeast species (Figure 3). Consequently, the antifungal test results performed with the agar dilution method were the same as the broth microdilution method, supporting the results of the broth microdilution method.

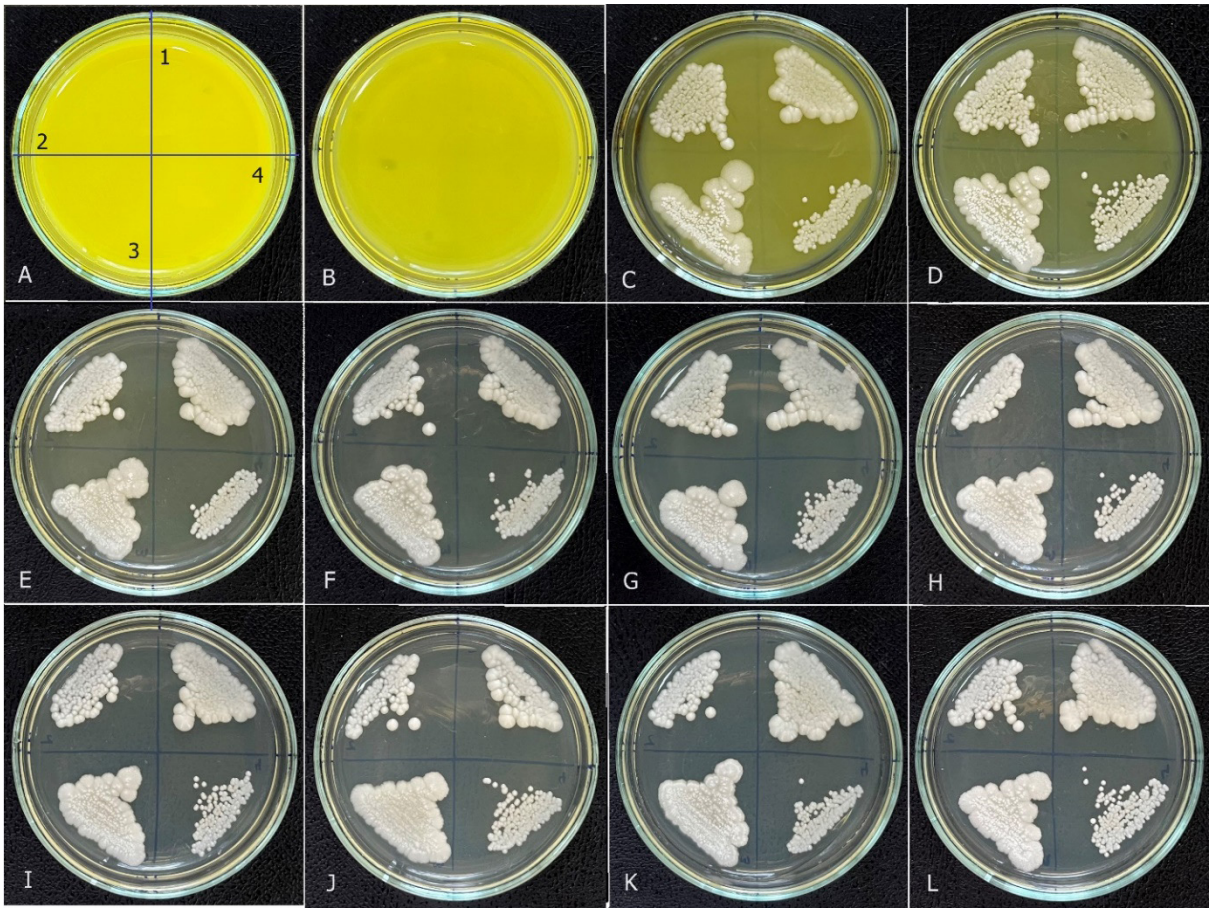

Figure 3. Plate pictures of Isorhamnetin antifungal assay against *C. tropicalis* (1), *C. albicans* (2), *C. krusei* (3), and *C. parapsilosis* (4) using sabouraud glucose agar. Plates from A to K include different concentrations of isorhamnetin (from 3.75 to 0.003 mg/mL). The plate L is positive control.

Flavonoids are natural polyphenolic compounds with two benzene rings flexibly attached to a heterocyclic pyrone ring. Flavonoids show antioxidative activity by inhibiting lipid peroxidation. Therefore, attention has been paid to the protective properties of flavonoids in various plants and foods. (Wattenberg, 1990; Mabry, 1980). Flavonoids constitute a significant portion of the metabolites in plant content and contain more than six thousand nutrients developed in various plants. Numerous studies have been conducted on the antioxidant, anti-allergic, anticancer, antimicrobial, and antiviral properties of flavonoids and their remarkable pharmacological components (Araujo et al., 2014; Li et al., 2014; Kim et al., 2011; Burda & Oleszek, 2001; Ramos, 2007).

Isorhamnetin is the metabolite of quercetin and an important methylated flavonoid due to its pharmacological and therapeutic properties (Khaled, 2020). Isorhamnetin has proven pharmacological effects in many areas. Isorhamnetin has anti-inflammatory and antioxidant properties and may inhibit the growth and metastasis of cancer cells (Wang

et al., 2018; Wei et al., 2018; Chi et al., 2016; Hu et al., 2015; Pengfei et al., 2009). Isorhamnetin plays an essential role in treating diseases through signaling pathways and cytokines. Pharmacologically, isorhamnetin has been evaluated in various studies to have therapeutic effects such as anti-pulmonary fibrosis, anti-osteoporosis, antioxidation, anti-hypoxia, anti-hyperuricemia, regulating immunity, anti-vitiligo and prevention of obesity (Gong et al., 2020; Khaled, 2020). Besides all these, a limited number of antimicrobial studies have been conducted with plant samples containing isorhamnetin. In a study with Kombucha focusing on polyphenolic content, isorhamnetin was associated with changes in bacterial membrane permeability and membrane morphology resulting from intracellular reactive oxygen species (ROS) formation. Isorhamnetin can penetrate the bacterial cell membrane through oxidative stress (Bhattacharya et al., 2018). The antibacterial property of isorhamnetin, which is found in the highest amount (28.79 µg/mg) in *Tamarix ramosissima* bark extract compared to other phenolic compounds, was proven by Ren et al. According to this study, the MIC value of *T. ramosissima* bark extract against bacterial species was found to be 5 mg/mL, and the minimum bactericidal concentration (MBC) value was 10 mg/mL. However, no antifungal activity was observed, and the mechanism of antibacterial activity was not explained (Ren et al., 2019). There appears to be a dilemma between this study and the antifungal study we did. This is because isorhamnetin was not used purely by Ren and his colleagues. Other phenolic compounds found in *T. ramosissima* may also induce the growth of fungal species. It has been stated that the *Ribes nigrum* L. leaf, which contains isorhamnetin in its chemical composition, is a useful antimicrobial, and the isorhamnetin obtained from this extract is bacteriostatic (Stević et al., 2010). In a study where isorhamnetin was found extensively in the plant flower of *Vernonia amygdalina*, the antibacterial activity was attributed to isorhamnetin (Habtamu & Melaku, 2018). However, until today, the studies were conducted with natural substances or plant extracts containing isorhamnetin in their phenolic content. There are very few antimicrobial activity studies with pure isorhamnetin, but these have been studied against a few specific species. The following studies are examples of works with pure isorhamnetin. The antifungal activity of isorhamnetin against *Aspergillus fumigatus*, which causes the serious ocular disease fungal keratitis, was evaluated, and it was observed that isorhamnetin inhibited *A. fumigatus* and reduced inflammatory factors (Tian et al., 2021). Another study using pure isorhamnetin investigated its antituberculosis activity against *Mycobacterium tuberculosis*. This study reported that isorhamnetin reduced IL-1β, IL-6, IL-12, and INF-γ levels in lung tissue and could be developed as a potent tuberculosis drug (Jnawali et al., 2016).

This study tested the antifungal activity of 97% pure isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and C. *parapsilosis* using agar dilution and broth microdilution methods. As a result of this study, it was found that isorhamnetin had a strong inhibitory effect against *Candida* species, and its MIC value was 1.875 mg/mL against all *Candida* species. The same study was conducted at the same concentrations against bacterial species: *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. However, no *in vitro* antibacterial effect of 97% pure isorhamnetin was observed at this concentration. Yeast cells are more resistant to antibiotic treatment than bacteria (Memişoğlu, 2019). However, in this study, the antifungal property of isorhamnetin may be attributed to the structural difference of the yeast cells. Consequently, it has been stated that isorhamnetin can be used as a natural therapeutic agent for fungal diseases. This preliminary study shows that isorhamnetin has antifungal activity instead of an antibacterial effect. It also shows that it can be used as an active ingredient against pathogenic opportunistic yeast species without harming the beneficial bacterial prebiotics in the human body. This preliminary study has proven the importance of isorhamnetin, whose pharmaceutical effectiveness has been proven in many medical fields, as a therapeutic or protective agent in infectious diseases.

The limitation of our study is that it was an *in vitro* study, and the effectiveness of isorhamnetin *in vivo* was not tested. Although many *in vitro* studies prove the pharmacotherapeutic properties of isorhamnetin in the literature, *in vivo* studies are limited. Finally, *in vivo* studies are needed to provide more data and clarify the exact mechanism of action of isorhamnetin so that it can be used clinically in treating diseases. The antifungal activity of isorhamnetin has not been tested against any other fungal species belonging to a different genus. However, it has been tested against *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis*, the species most common in fungal diseases, and it was observed that isorhamnetin has a significant inhibitory effect against fungal species. Another limitation of our study is that the inhibitory mechanism of isorhamnetin against *Candida* species has yet to be explained, and a detailed study, perhaps at the molecular level, is needed. However, especially considering the antifungal drug resistance that has occurred in recent years and the severe side effects of synthetic antifungals, proving the antifungal properties of isorhamnetin produces promising results. This preliminary study proved the importance of isorhamnetin as a therapeutic or preventive pharmaceutical agent in infectious diseases.

CONCLUSION

Isorhamnetin is a valuable flavonoid and therapeutic compound with proven healing activity in the medical and pharmacological fields. In the last decade, many studies have been conducted on the anti-osteoporosis, antioxidant, anti-hypoxia, anticancer, and anti-hyperuricemia properties of isorhamnetin. This study is the first to prove the antifungal activity of pure isorhamnetin against *C. tropicalis, C. albicans, C. krusei,* and *C. parapsilosis* (MIC: 1.875 mg/mL). This preliminary study raises the possibility that isorhamnetin can be used as a preventive or therapeutic pharmaceutical agent in infectious *Candida* diseases.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

Tuba Unver conceived the principal idea, designed and carried out the experiment, wrote the original draft, and took all responsibility for the manuscript.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Forecasting some climate parameters of Türkiye using the SSP3-7.0 scenario for the years 2040–2059

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Abstract
This study employs the Coupled Model Inter-comparison Projects (CMIPs) and the Sixth phase of CMIPs (CMIP6) to unravel the multifaceted impacts of global climate change on climate of Türkiye. The CMIP6 data, fundamental to the Intergovernmental Panel on Climate Change (IPCC) Assessment Reports, forms the basis for projecting future climate scenarios, specifically under the medium-high reference scenario SSP3-7. Utilizing a suite of global climate models, including the innovative Multi-Model Ensemble (MME) approach, this study combines predictions to enhance the precision climate projections of Türkiye. Historical data spanning from 1951 to 2020 were subjected to rigorous statistical analysis, including descriptive statistics and regression analysis. The findings reveal an unequivocal upward trajectory in Türkiye's annual mean temperature, with an accelerated pace in recent decades. Despite a lack of a significant long-term trend in annual precipitation from 1951 to 2020, the rate of change in precipitation is accelerating, indicating potential future challenges. Projections for 2040-2059 under the SSP3-7.0 scenario indicate a non-uniform increase in mean temperature across Türkiye, with the southern and western regions facing the most significant impact. This warming trend poses imminent threats to agriculture, altering crop yields and increasing the risk of heat stress for livestock. Additionally, the projected decrease in precipitation, alongside a surge in hot days and tropical nights, underscores the urgency for adaptive measures. As Türkiye navigates the complex terrain of climate change, this study provides valuable insights, emphasizing the significance of robust climate modeling for informed decision-making. The results underscore the imminent challenges Türkiye faces and emphasize the critical importance of proactive climate action on both national and global fronts.
Keywords: Drought, Changing climate conditions, Precipitation

INTRODUCTION

The rigorous rise in global temperatures, a hallmark of the ongoing climate crisis, has profound implications for regions worldwide. Temperature increases might have a big impact on Turkey as well, which is particularly vulnerable to the effects of climate change because of its location in the Mediterranean basin. According to Tokuşlu (2022) there is a high probability that the warming in this region will surpass the global average, potentially having negative consequences for the nation's agricultural, water resources, and biodiversity.

Under the medium-high reference scenario, SSP3-7, which envisions sustained economic growth alongside increasing inequality and environmental degradation, Türkiye faces a complex web of challenges. Projected trends, derived from a suite of global climate models, indicate a surge in global population,

economic growth, and fossil fuel use, with agriculture and urban development dominating land use. The Multi-Model Ensemble (MME) approach improves the accuracy of future climate predictions by combining predictions from different models.

The study by Acar et al. (2018) examines daily minimum and maximum temperatures from 156 weather stations in order to analyze the long-term changes in temperature extremes in Turkey. The study looks into trends in hot and cold days using Mann-Kendall trend analysis and cluster analysis. It finds that the frequency of hot days is rising while that of cold days is falling. The frequency of extremely hot days has increased since 2000, while the frequency of extremely cold days has decreased since 2005. The warmest year on record was 2010. Analyzing historical data from 1951 to 2020, this current study unveils an unequivocal upward trajectory in Türkiye's annual mean temperature. Paradoxically, annual precipitation exhibits an accelerating rate of change over recent decades, despite an overall lack of a significant long-term trend. This enigma underscores the complexity of climate change impacts on precipitation patterns, hinting at potential future challenges.

The forthcoming projections for 2040-2059 paint a concerning picture. Türkiye anticipates a rise in mean temperature, unevenly distributed across regions, posing imminent threats to agriculture and livestock. The projected decrease in precipitation, coupled with an increase in hot days and tropical nights, particularly in the Aegean and Mediterranean regions, underscores the urgency of adaptive measures.

This study delves into the climate projection data, sourced from the Coupled Model Inter-comparison Projects (CMIPs), to comprehend the impacts of climate change on Türkiye. The CMIP6 data, a pivotal component of the IPCC Assessment Reports, provides a comprehensive foundation for assessing the future climate scenarios (Hoegh-Guldberg et al., 2019). This study aims to understand the potential effects of these changes on Türkiye by examining climate change scenarios between 2040-2059. Moreover, as the world grapples with the consequences of anthropogenic climate change, this study not only sheds light on the impending challenges for Türkiye but also underscores the importance of robust climate modeling for informed decision-making on both national and global scales.

MATERIALS AND METHODS

Data selection for climate projection

Climate projection data is modeled data from the global climate model compilations of the Coupled Model Inter-comparison Projects (CMIPs), overseen by the World Climate Research Program. Data presented is CMIP6, derived from the Sixth phase of the CMIPs. The CMIPs form the data foundation of the IPCC Assessment Reports. CMIP6 supports the IPCC's Sixth Assessment Report. Projection data is presented at a 1.0º x 1.0º (100km x 100km) resolution. The Climate Change Knowledge Portal (CCKP) continues to add new, additional indicators as they are produced and as appropriate.

Selection of scenario

SSP3-7 is a medium-high reference scenario that assumes continued economic growth and technological development, but also increasing inequality and environmental degradation. SSP3-7 is based on a set of assumptions about the future of global population, economic growth, energy use, and land use (Fujimori et al., 2017). These assumptions are used to drive a suite of global climate models to simulate the future climate. The specific assumptions of SSP3-7 are as follows:

- Global population reaches 10.8 billion by 2100.

- Global Gross Domestic Product (GDP) per capita increases by 2.5% per year.

- Fossil fuel use continues to increase, but renewable energy use also increases.

- Land use is dominated by agriculture and urban development.

Model selection

In Multi-Model Ensemble (MME) the predictions of each model are individually calculated and then combined using a variety of methods. The most common method is to simply average the predictions of all models (Kug et al., 2008). The choice of MME method depends on the specific application and the available data.

Historical data selection and Statistical analysis

The historical data used in this study for the period 1951 to 2020 was sourced from the CCKP. This portal provides comprehensive climate data, including temperature and precipitation records, which are critical for our analysis. Utilizing this data, the historical reference period was set from 1995 to 2014 to ensure a robust foundation for accurate regression modeling. The MME approach, incorporating CMIP6 models, was applied to process this data. This

included a detailed examination of climate patterns over the years, allowing for nuanced insights into the climatic changes occurring in Türkiye. The regression model used in this study for analyzing climate data is a statistical tool designed to understand and forecast climate trends in Phyton. This model examines the relationship between various climate parameters, such as temperature and precipitation, over time. By incorporating historical data, the model identifies patterns and trends, which are then used to make projections about future climate conditions. The strength of this model lies in its ability to handle large and complex datasets, providing accurate and reliable forecasts. The methodology involves analyzing historical climate data to establish baseline trends, upon which future projections are built. This approach is particularly effective in understanding the impacts of climate change in specific regions like Türkiye. This process involved the following steps:

Data Collection: Historical climate data from 1951 to 2020 was obtained from the CCKP.

Variable Selection: Temperature and precipitation were selected based on their relevance to the study's objectives.

Model Specification: A regression model was formulated to establish relationships between these variables over time.

Data Processing: The collected data was pre-processed for consistency and accuracy before being fed into the model.

Model Fitting: The regression model was fitted to the historical data, allowing for the identification of significant climate trends and patterns.

Projection and Analysis: The model was then used to project future climate scenarios, providing insights into potential changes in Türkiye's climate under various conditions.

RESULTS

Current Datas

Annual Mean Temperature Trends in Türkiye (1951-2020)

The annual mean temperature for the years 1951–2020 and temperature trend lines for different time periods in Türkiye is shown in Figure 1. This graphic depiction has four different trend lines, each of which stands for a different time period:

Average Annual Temperature: This line represents Türkiye's average annual temperature, offering a baseline for comparison with trend lines.

Trend 1951-2020: Trend from 1951 to 2020: This trend covers about 70 years. Over this time period, the average temperature has increased, as indicated by the positive slope. The rate of warming is represented by the slope's magnitude. Global greenhouse gas emissions have increased, there has been a rise in industrial activity, and fossil fuel use has become more intensive between the 1950s and 2020. These factors may have aided in the process of global warming and the rise in mean air temperatures that followed. The trend's slope is roughly 0.0214°C annually, resulting in a 13.11% increase in temperature over the course of the 70 years.

Trend 1971-2020: Trend from 1971 to 2020: This trend, which covers about 50 years, likewise has a positive slope. There's a chance that this period's warming rate will be different from the 1951–2020 period's. The effects of global warming intensified during this period, and scientific research and public awareness of climate change increased. The slope for this time span is roughly 0.0427°C annually, meaning that over a 50-year span, the temperature increased by 18.95%.

Trend 1991-2020: Trend from 1991 to 2020: This trend, which covers the shortest amount of time—roughly 30 years—may point to a quicker rate of global warming than the two periods before it. The acceleration of climate change and the rise in extreme weather events during this time, particularly in the last ten years, make it noteworthy. This period's rapid warming may be directly linked to rising greenhouse gas emissions and atmospheric concentrations of those gases. The slope for this time span is roughly 0.0689°C annually, resulting in an 18.51% rise in temperature over the course of 30 years.

The information shown in Figure 1 clearly shows that Türkiye's annual mean temperature has been rising since 1951. The rate of this warming trend has increased during the past few decades. In particular, the trend line for 1971–2020 indicates a more marked increase at 0.4°C per decade, whereas the trend line for 1951–2020 reveals an annual increase of 0.2°C. The most recent trend line shows the fastest rate of increase, at 0.4°C per decade, and spans the years 1991 to 2020.

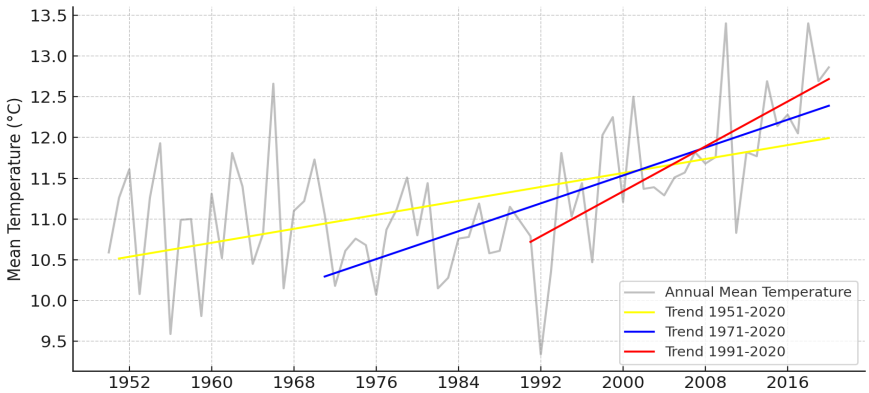

Figure 1. Mean temperature Annual Trends (from 1951 to 2020) with Significance of Trend per Decade, Türkiye.

Annual Precipitation Trends in Türkiye (1951-2020)

Figure 2 presents annual trends in precipitation in Türkiye for the period 1951-2020. The data reveals no significant trend in annual precipitation during this period. However, a noteworthy observation is the acceleration in the rate of change in annual precipitation over recent decades. The trend from 1971-2020 shows a slight decrease, while the trend from 1991-2020 displays a slight increase in annual precipitationIt can be surprising that there isn't a clear long-term trend (1951-2020) considering the predicted influence of climate change on precipitation patterns. However, it is important to note that the trend lines show that the rate of change in annual precipitation has been accelerating in recent decades. This suggests that the effects of climate change on precipitation patterns may become more pronounced in the future. The lack of a significant trend in annual precipitation in Türkiye over the period 1951-2020 does not mean that climate change is not having an impact on precipitation patterns. Climate change is causing changes in the intensity and frequency of precipitation events, as well as the timing of precipitation events. These changes are already being felt in some parts of Türkiye, and they are likely to become more pronounced in the future. For instance, Kara et al. (2016) noted a significant increase in extreme precipitation events for Istanbul, suggesting a future rise in flooding risks. Complementing this, Danandeh Mehr et al. (2020) observed an expected decrease in drought events for Ankara, indicating variations in drought patterns due to changing precipitation regimes. Sen et al. (2012) also highlighted potential increases in temperature and decreases in precipitation, leading to severe drought conditions, particularly in southwestern Türkiye. These findings collectively underscore the ongoing and future challenges posed by climate change to water resource management across different Türkiye regions.

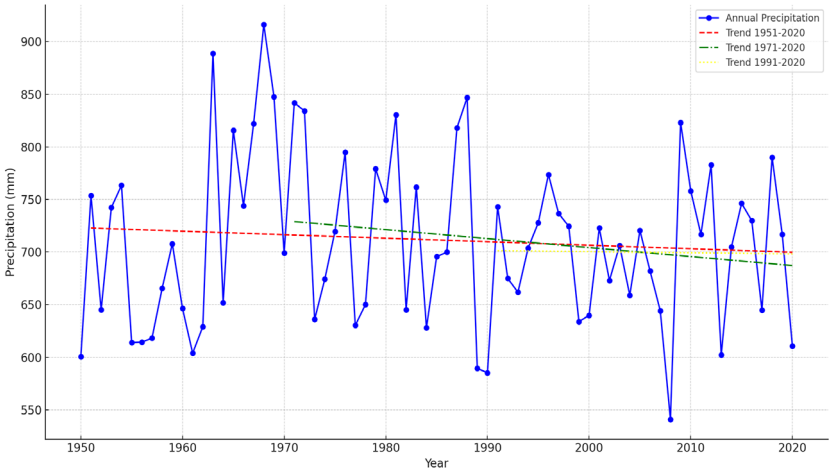

Figure 2. Precipitation Annual Trends (from 1951 to 2020) with Significance of Trend per Decade, Türkiye

Annual Number of Tropical Nights (>20°C) Trends in Türkiye (1951-2020)

Figure 3 shows the annual number of tropical nights (Tmin > 20°C) in Türkiye from 1951 to 2020, along with the significance of the trend per decade. The number of tropical nights has increased significantly over the past 70 years, with an average increase of 1.1 nights per decade. The trend has been particularly pronounced since the 1990s, with an average increase of 2.2 nights per decade.

This increase in tropical nights is likely due to a combination of factors, including climate change, urbanization, and changes in land use. Climate change is causing global temperatures to rise, which is leading to more frequent and intense heat waves. Urbanization also contributes to heat waves, as urban areas tend to be warmer than surrounding rural areas. Changes in land use, such as deforestation, can also contribute to heat waves by reducing the amount of shade and vegetation, which can help to cool the air.

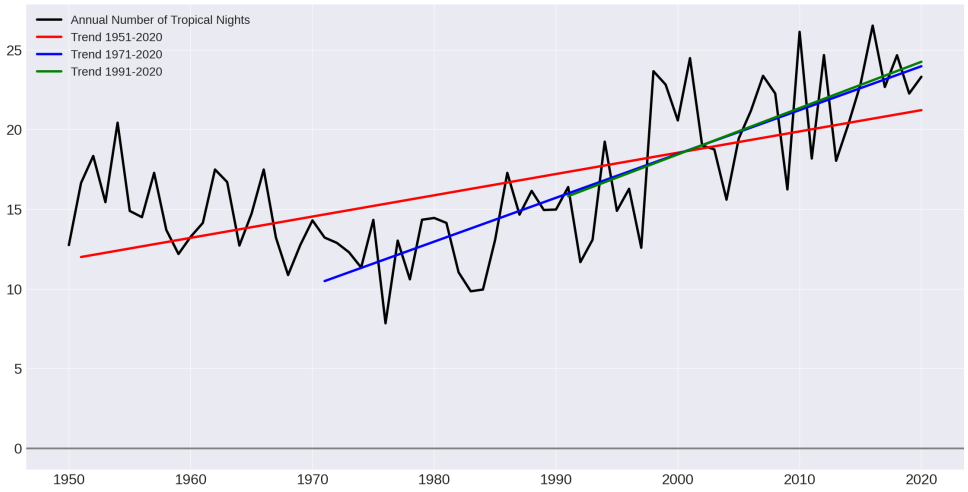

Figure 3. Number of Tropical Nights (Tmin>20°C) Trends (from 1951 to 2020) with Significance of Trend per Decade, Türkiye

Projected Datas

Projected Mean temperature (2040-2059)

The Figure 4 shows the projected mean temperature for Türkiye in the period 2040-2059. The graph is divided into two parts:

(a) Graph: This graph shows the predicted average temperature for Türkiye in general. Black circles line represent historical mean temperature for the period 1995-2014, and the shaded area represents the uncertainty range under the SSP3-7.0 scenario. Red rectangles line represent the average temperature predicted for Türkiye in the 2040-2059 period under the SSP3-7.0 scenario.

(b) Map: This map shows the projected mean temperature for different regions of Türkiye. The colors on the map represent the change in mean temperature, relative to the historical reference period (1995-2014).

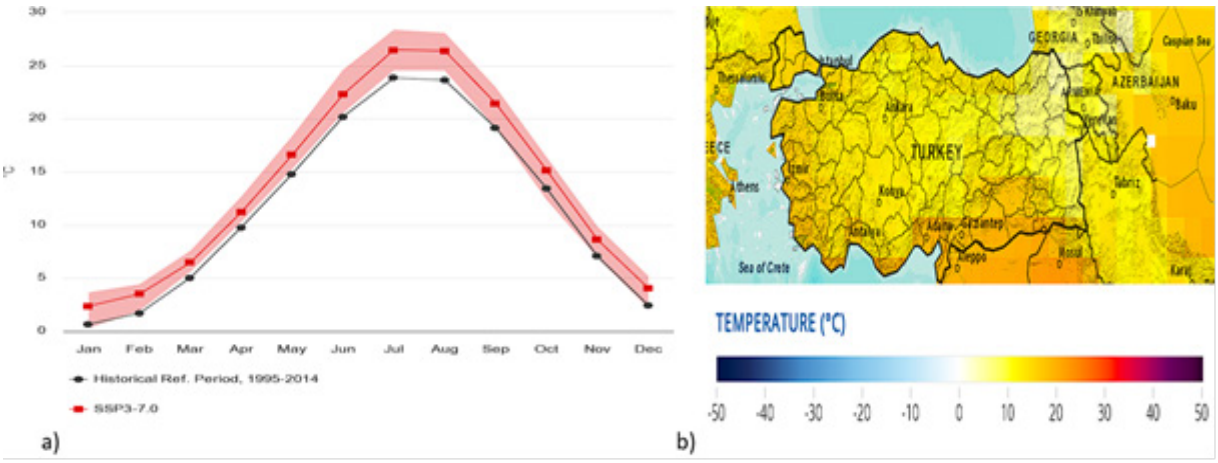

Figure 4. Projected Climatology of Mean-Temperature for 2040-2059 (Annual Türkiye; (Ref. Period: 1995-2014), SSP3-7.0, Multi-Model Ensemble as a graph (a) and regional map (b)

The graph shows that the historical mean temperature for Türkiye is 13.5°C. The range of uncertainty under the historical mean temperatureis from 13.2°C to 13.8°C. The red rectangles line shows that the projected mean temperature for Türkiye in the period 2040-2059 is 15.0°C, which represents an increase of 1.5°C. This is within the range of uncertainty for the historical mean temperature under the SSP3-7.0 scenario.

The map (Figure 4 (b)) shows that the projected increase in mean temperature is not uniform across Türkiye. The southern and western regions are projected to experience the largest increase in mean temperature, with increases of up to 2.0°C. The northeastern and eastern regions are projected to experience a smaller increase, with increases of up to 1.0°C.

The projected increase in mean temperature is likely to have a significant impact on agriculture in Türkiye. The warmer temperatures are likely to lead to changes in crop yields, and they are also likely to increase the risk of heat stress for livestock.

Projected Precipitation (2040-2059)

The Figure 5 shows the projected monthly average precipitation for Türkiye in the period 2040-2059, under the SSP3-7.0 scenario. The black circles represent the historical average precipitation for the period 1995-2014, and the shaded area represents the range of uncertainty under the SSP3-7.0 scenario. The red rectangles represent the projected average precipitation for Türkiye in the 2040-2059 period under the SSP3-7.0 scenario.

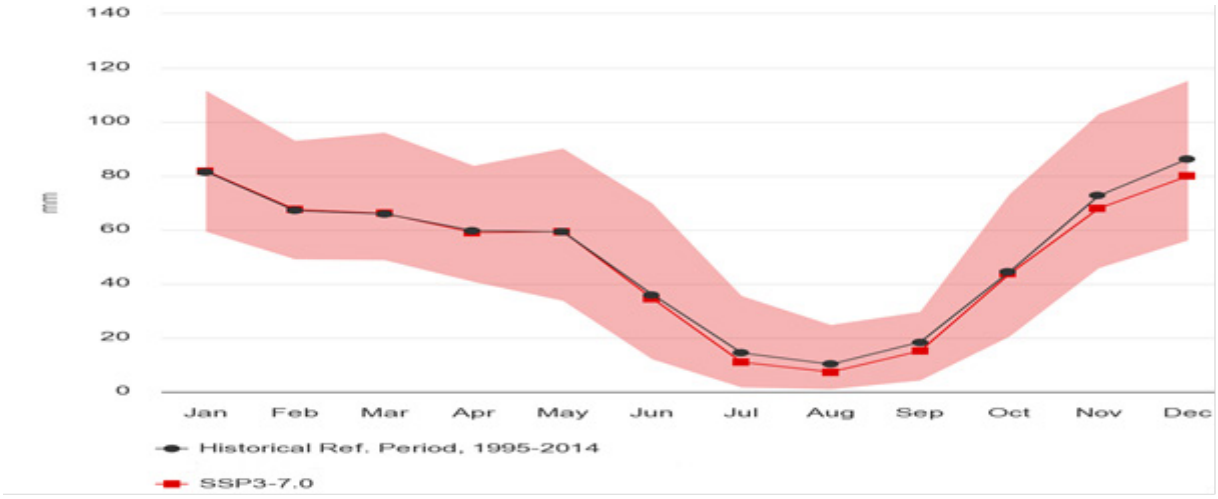

Figure 5. Projected Climatology of Precipitation for 2040-2059 Türkiye; (Reference Period: 1995-2014), SSP 3-7.0, Multi-Model Ensemble

The graph indicate that the projected average precipitation for Türkiye in the 2040-2059 period is significantly lower than the historical average precipitation. The range of uncertainty is from -10% to -20%. This means that there is a high likelihood that Türkiye will experience a significant decrease in precipitation in the coming decades. The projected decrease in precipitation is likely to have a significant impact on agriculture in Türkiye. The aridconditions are likely to lead to changes in crop yields, and they are also likely to increase the risk of droughts.

Number of Projected Hot Days (Tmax>30°C)

The Figure 6 shows a significant increase in the projected number of hot days, from an average of 10.4 days per year in the reference period to an average of 21.6 days per year in the 2040-2059 period. This represents an increase of 107%.

The increase in hot days is expected to be particularly pronounced in the southeastern region (Figure 6b) of Türkiye, where the number of hot days is projected to increase by up to 150%.

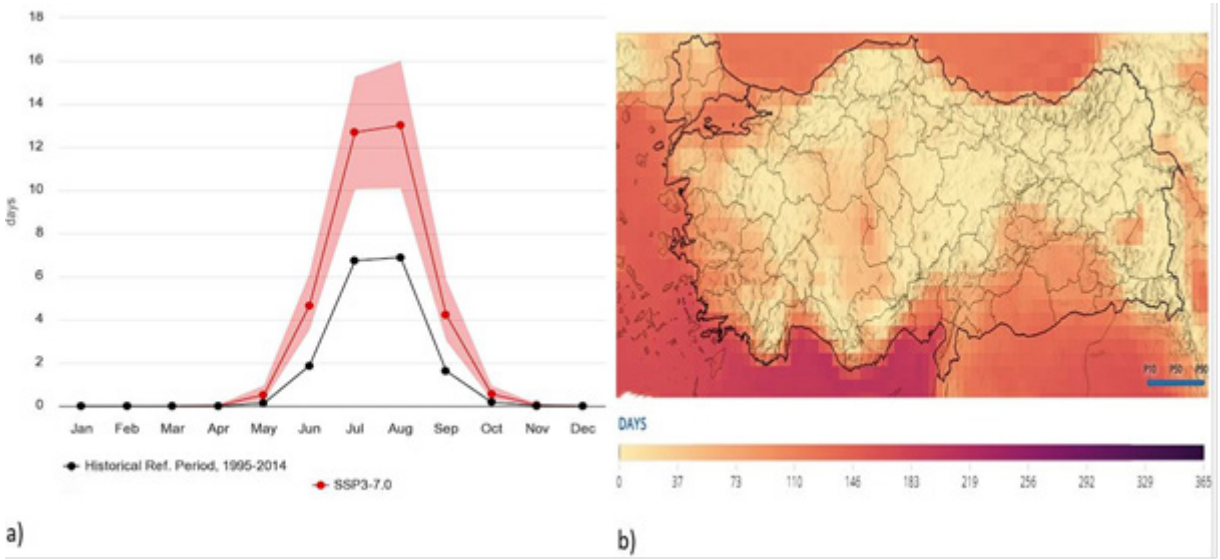

Figure 6. Projected Number of Hot Days with monthly graph (a) and map (b) (tmax>30oC) for 2040-2059 Türkiye; (Reference Period: 1995-2014), SSP 3-7.0, Multi-Model Ensemble

Number of Tropical Nights (Tmin>20°C)

The Figure 7 (a) shows the projected monthly number of tropical nights (Tmax>30°C) for 2040-2059 under SSP 3-7.0, Multi-Model Ensemble. The Figure 7 (b) shows the spatial distribution of the projected increase in the number of tropical nights.

According to the graph, the projected number of tropical nights is expected to increase by 10-20 nights per year by 2040-2059. The increase is expected to be most pronounced in the Aegean and Mediterranean regions.

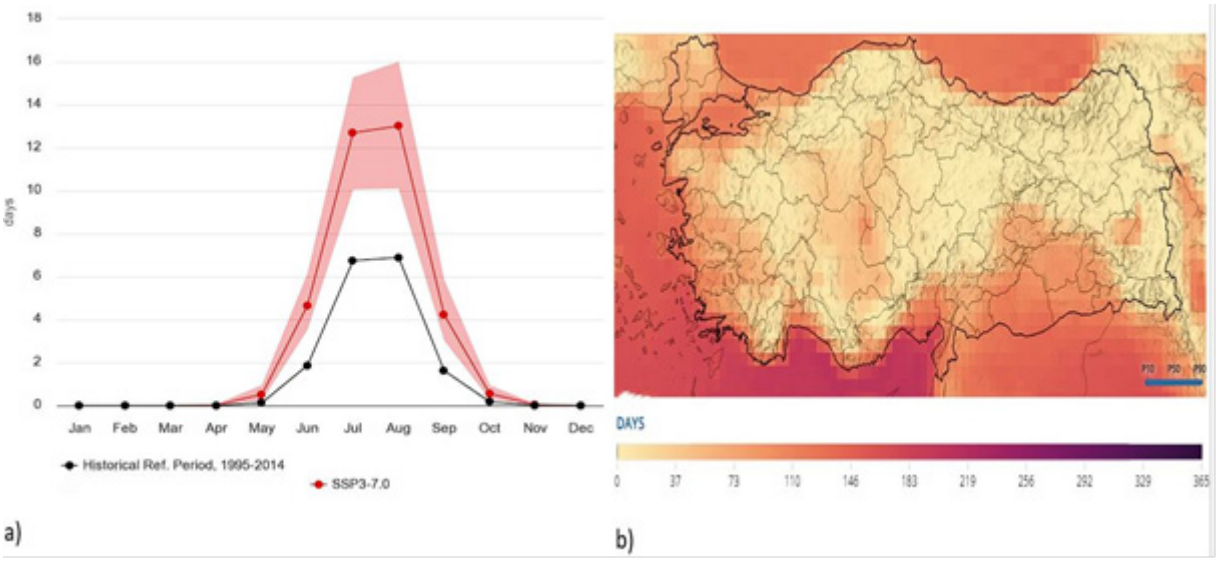

Figure 7. Projected Number of Tropical Nights with monthly graph (a) and map (b) (tmax>30°C) for 2040-2059 Türkiye; (Reference Period: 1995-2014), SSP 3-7.0, Multi-Model Ensemble

DISCUSSION

Our study's conclusions are consistent with the current state of knowledge regarding the various effects of climate change in Turkey. The impacts of climate change on agricultural productivity and water resources in various regions of Turkey are described in detail in studies conducted by Acar et al. (2018) and Bağçaci et al. (2021). More precisely, Bektaş and Sakarya (2023) emphasize the expected rise in fluctuations in temperatures and the frequency of extreme weather events. The adverse impacts of climate change on water resources and agricultural incomes are documented in the works of Demircan et al. (2017) and Pilevneli et al. (2023). Similarly, the impacts on Türkiye's water resources are the main focus of Rustem and Goknar (2022). These studies emphasize how crucial it is to create strategies and policies to comprehend and prepare for the regional and global effects of climate change. In this regard, our research advances our knowledge of the detailed and complex effects of climate change in Turkey and enhances the discussion currently taking place in this area.

The observed temperature rise aligns with the global phenomenon of climate change, supported by the Intergovernmental Panel on Climate Change's assertion that human activities contribute significantly to the increase in global mean surface temperature (Masih, 2018). Bozoglu et al. (2019) argue that climate change is already having a significant impact on agriculture in Türkiye. They cite evidence of increased crop yields in some regions, but also of decreased yields in other regions due to drought, heat stress, and pests. Pilevneli et al. (2023) investigate the impact of climate change on agricultural production and incomes in 25 river basins in Türkiye. They find that climate change is likely to lead to a decrease in agricultural production and incomes in most river basins, with the most severe impacts being felt in the southeastern region of the country. These studies suggests that while global trends are evident, local variations are significant and should be considered in policy and adaptation strategies.

Some recent studies, also, project a 1.4°C average temperature increase in Türkiye (Tokuslu, 2022), with anticipated rises up to 2-3°C (Rustem and Günal, 2022). These changes have direct implications for extreme weather events, such as droughts, floods, and heatwaves (Bektaş and Sakarya, 2023). As for projected temperature increase estimates for Türkiye, the projected increase of 1.5°C aligns with findings from Oruc et al., (2019), which investigates the impact of climate change on extreme precipitation in Türkiye. They emphasizes the consequences of non-stationary climate patterns and the potential intensification of extreme weather events due to climate change. Every single one of these projections is in line with the current study. This agreement emphasizes how urgent it is to address the effects of climate change, particularly with regard to anticipating and reducing extreme weather events.

Climate models indicate a general decrease in annual precipitation in Türkiye, exacerbating water resource challenges. To address these issues, proactive measures like green initiatives and resilient infrastructure are crucial (Demircan et al., 2017). The projected decrease in precipitation aligns with findings from Andrade et al., (2021), which focuses on climate change projections of aridity conditions. Andrade's research emphasizes the likelihood of significant impacts due to arid conditions in certain regions. The projected range of -10% to -20% in precipitation is consistent with

these concerns. Moreover, the projected reduction in yearly precipitation, as suggested by climate models and the study conducted by Andrade et al. (2021), enhances the difficulties related to water resources in Turkey. In line with these concerns, the anticipated range of -10% to -20% in precipitation requires comprehensive management of water resources that takes socioeconomic and climatic factors into account. In order to proactively address these new challenges, it is crucial that green initiatives and resilient infrastructure be prioritized, as stressed by Demircan et al. (2017).The results of other studies are in line with the anticipated rise in the number of tropical nights in Turkey. The observed increase in the number of tropical nights corresponds with findings from previous studies on climate change projections in Türkiye. Demircan et al., (2017) highlights a significant rise in mean temperatures, which is consistent with the increase in tropical nights projected here. This aligns with the broader understanding of climate change leading to more extreme temperature conditions in the region. The projected increase of 10-20 tropical nights per year is most pronounced in the Aegean and Mediterranean regions. These regions are known for their warm climate, and this increase is expected to exacerbate the already warm conditions. To continue in greater detail, it is clear that adaptation and mitigation strategies for climate change in Türkiye must be customized to the unique regional characteristics of the phenomenon. As our study has shown, there are serious concerns regarding the rise in tropical nights for human health, agriculture, and ecosystems especially in the warmer regions of the Mediterranean and Aegean. Sen et al. (2012) and Tayanc et al. (2009) findings are consistent with the larger understanding of climate change causing more extreme temperature conditions in the region. A targeted strategy is required to address the particular challenges posed by higher nighttime temperatures because of the predicted increase of 10–20 tropical nights annually, which will be most noticeable in these regions.

CONCLUSION

The comprehensive analysis of current and projected climate data for Türkiye underscores the imminent challenges posed by climate change. The observed upward trajectory in annual mean temperatures, especially the accelerated increase since the 1990s, aligns with global climate change patterns. This temperature rise, supported by multiple studies, is anticipated to have significant implications for agriculture, with potential changes in crop yields and heightened risks of heat stress for livestock.

Contrastingly, the analysis of annual precipitation trends reveals a paradoxical lack of a significant long-term trend (1951-2020) in Türkiye. Despite this, the acceleration in the rate of change in recent decades suggests that the effects of climate change on precipitation patterns may intensify in the future. Projections indicate a significant decrease in average precipitation for the period 2040-2059, with a range of uncertainty from -10% to -20%. This projected decrease poses substantial risks to agriculture, leading to potential changes in crop yields and an increased risk of droughts.

The projections for the number of hot days (Tmax>30°C) and tropical nights (Tmin>20°C) further emphasize the intensification of extreme weather events. The substantial increase in hot days, particularly in the southeastern region, aligns with the broader understanding of climate change-induced heatwaves. The rise in tropical nights, concentrated in the Aegean and Mediterranean regions, poses additional challenges to human health, agriculture, and ecosystems.

In conclusion, the synthesis of current and projected data accentuates the urgency for comprehensive strategies and proactive measures in Türkiye. Addressing the impacts on agriculture, water resources, and human well-being necessitates green initiatives, resilient infrastructure, and concerted efforts to mitigate and adapt to the evolving climate patterns.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review
Externally peer-reviewed.

Conflict of interest
No conflict of interest exists.

Author contribution
Each process in the article was conducted by Eser Celiktopuz (EC).

Ethics committee approval
Ethics committee approval is not required.

Funding
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Data availability
Not applicable.

Consent to participate
Not applicable.

Consent for publication
Consent for publication to the manuscript should be specified in this section

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RESEARCH ARTICLE

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Determining individuals' knowledge, attitudes, and experiences concerning sumac (*Rhus coriaria* L.)

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Abstract
The aim of this study is to determine the individuals' knowledge, attitudes, and experiences concerning sumac (*Rhus coriaria* L.). The researchers collected the data from 139 participants, living in the cities of Siirt and Mardin, by using a survey, prepared based on the relevant literature, between 01 July and 15 August 2021. The mean age of the participants was 24.42±4.44 (min:18 – max:65) and 82.0% of them were female. 45.3% of the participants had an undergraduate or higher education. 81.3% of the participants stated that they had knowledge about the health benefits of sumac. 74.1% of them stated that they obtained this information from their family, partner, friend, and relatives. When the frequency of consuming sumac was evaluated, 28.1% of the participants stated that they consumed it once a week and 20.9% every day. 80.6% of the participants stated that sumac was effective for the treatment of stomach disorders, 79.9% for the treatment of infection, 74.1% for the treatment of influenza, 67.6% for alleviating respiratory distress, 63.3% for the treatment of gastric ulcer, and 59.7% for lowering blood pressure. Based on the experiences of the individuals, it was determined that sumac was effective for the treatment of stomach disorders (80.6%), the treatment of infection (79.9%), the treatment of influenza (74.1%), alleviating respiratory distress (67.6%), the treatment of gastric ulcer (63.3%), and lowering blood pressure (59.7%).
Keywords: Sumac, Rhus Coriaria L., Knowledge, Attitude, Experience

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INTRODUCTION

Rhus coriaria L. (Anacardiaceae), locally known as Sumac, is gathered in autumn. It is used in the form of powder or boiled with a certain amount of water. The plant is used in two forms: fruit and leaf (Yücel et al., 2011). *Rhus coriaria* is the only Rhus species grown in Turkey. It is distributed in the Mediterranean, Aegean, Southeastern Anatolia, Northern Anatolia, Thrace, and Central Anatolia regions in Turkey (Koyuncu and Köroğlu, 1991).

Sumac, which is mostly used as a culinary spice, has also been employed in traditional medicine to treat various illnesses such as liver diseases, diarrhoea, urinary problems, gastric ulcers, dysentery, haemorrhoid, and gout and is also often utilised for healing wounds and lowering blood glucose, cholesterol, and uric acid levels (Sakhr & El Khatib, 2020; Candan, 2003; Rayne & Mazza, 2007; Shabbir, 2012). It has been reported that sumac has antibacterial, antiviral, antifungal, anticandidal, antiulcer, antiseptic, and antihepatoxic effects due to gallic and ellagic acid, quercetin, isoquercetin, isoquercetin myricetin, myricitrine, and tannin in its composition (Candan, and Sökmen, 2004; Gıancarlo et al., 2006; Gülmez et al., 2006). The studies have revealed that R. coriaria is antimicrobial, antifungal, antiviral (Rayne & Mazza, 2007), antioxidant (Bozan et al., 2003), anti-

inflammatory (Panico et al., 2009), hepatoprotective (Pourahmad et al., 2010), and cardiovascular protective effects (Beretta et al., 2009) have important biological effects. Many previous in vitro and in vivo animal and human studies revealed the potential of sumac to lower blood glucose (Gupta et al., 2005; Ahangarpour et al., 2014; Shidfar et al., 2014; Abedi Gaballu et al., 2015; Salimi et al., 2015; Pourahmad et al., 2010).

Rhus coriaria fruit contains a wide range of bioactive compounds that can be beneficial to humans and are active in wound healing processes. A mouse study reported that cream formulated from sumac treated excision and burn wounds more effectively than commercial creams (Alsarayreh et al., 2021). As a result of the literature review, no article on the experiences of the people regarding the use of sumac was found. This study aims to fill this gap in the literature.

In the light of this information, this study was conducted to determine the knowledge, attitudes, and experiences of individuals living in the cities of Mardin and Siirt concerning sumac (*Rhus coriaria* L.).

MATERIALS AND METHODS

This cross-sectional study was conducted to determine the knowledge, attitudes, and experiences of individuals concerning sumac.

Everyone who could be reached within the specified time period was included in the sample without any sampling method. The sample consisted of individuals between the ages of 18-69 who were actively using the internet-social media (E-Mail, WhatsApp, Instagram, Facebook etc.), were living in the cities of Siirt and Mardin, and voluntarily agreed to participate in the study.

The researchers collected the data from 139 individuals who were living in the cities of Siirt and Mardin and using actively internet-social media (E-Mail, WhatsApp, Instagram, Facebook etc.) by using a survey prepared based on the relevant literature between 01 July and 15 August 2021.

The data collection form consists of 9 questions including demographic questions such as gender, age, etc., 4 questions including the use of sumac, frequency of use, having information about sumac, etc., and 1 open-ended question that determines the experiences of individuals who examine sumac crops. The second part consists of 10 questions about what sumac is effective in line with the literature (Table1, Table2, Table3).

Ethical Considerations

Ethics committee approval was obtained from Gümüşhane University Scientific Research and Publication Ethics Committee in order to conduct the study.

Statistical Analysis

Means, median, frequencies, and percentage were used as descriptive statistics to show the results.

RESULTS

The mean age of the participants in the study was 24.42±4.44 (min:18 – max:65) and 82.0% of them were female. 45.3% of the participants had an undergraduate or higher education, and 70.5% stated that they were unemployed. More than half of the individuals (69.1%) reported that their income level was moderately high. More than half of the participants (62.6%) stated that their health status was good (Table 1).

As a result of the statistical analysis, it was found that there was a significant difference only between gender and sumac use among sociodemographic characteristics and gender and sumac use was more common among women ($p<0,001$).

All of the participants stated that they used natural or herbal approaches. 81.3% of the participants stated that they knew about the health benefits of sumac. 74.1% of them stated that they obtained this information from their family, partner, friend, and relatives. When the frequency of consuming sumac was evaluated, 28.1% of the participants stated that they consumed it once a week and 20.9% every day (Table 2).

The benefits of sumac were evaluated based on the own experiences of those who used sumac. 80.6% of the participants stated that sumac was effective for the treatment of stomach disorders, 79.9% for the treatment of infection, 74.1% for the treatment of influenza, 67.6% for alleviating respiratory distress, 63.3% for the treatment of gastric ulcer, and 59.7% for lowering blood pressure (Table 3).

Table 1. Socio-demographic characteristics

	n	%
Gender		
Female	114	82.0
Male	25	18.0
Educational background		
Illiterate	3	2.2
Primary school	7	5.0
Secondary school	13	9.4
High school	53	38.1
Undergraduate and higher	63	45.3
Employment status		
Yes	41	29.5
No	98	70.5
Occupation		
Civil-servant	15	10.8
Self-employed	11	7.9
Worker	15	10.8
Unemployed	98	70.5
Income level		
Low	28	20.1
Middle	96	69.1
High	15	10.8
Longest lived place of residence so far		
Village/Town	12	8.6
Province	32	23.0
District	95	68.3
How do you define your health?		
Poor	2	1.4
Moderate	50	36.0
Good	87	62.6

Table 2. Characteristics related to the use of sumac

	n	%
Do you have knowledge about the health benefits of sumac products?		
Yes	113	81.3
No	26	18.7
If so, where did you obtain this information from?		
Radio- Television	39	28.1
Newspaper – Magazine	13	9.4
Healthcare professionals	17	12.2
Family, Partner, Friend, Relative	103	74.1
Other	6	4.3
Frequency of consuming sumac		
Every day	29	20.9
Once a week	39	28.1
Once every 15 days	15	10.8
Rarely	56	40.3

Table 3. Experiences of sumac users

	Sumac			
	Effective		Ineffective	
	n	%	n	%
Alleviating respiratory distress	94	67.6	45	32.4
Stomach disorders	112	80.6	27	19.4
Lowering blood pressure	83	59.7	56	40.3
As an antiseptic	72	51.8	67	48.2
As a diuretic	70	50.4	69	49.6
Periodontal diseases	71	51.1	68	48.9
As a blood glucose regulator	59	42.4	80	57.6
Treatment of influenza	103	74.1	36	25.9
Treatment of infection	111	79.9	28	20.1
Gastric ulcer	88	63.3	51	36.7

DISCUSSION

Rhus coriaria (RC), commonly known as sumac, is a medicinal spice known for its anti-lipidemic, anti-fibrogenic, anti-inflammatory, antidiabetic, antioxidant, anti-ischemic, antithrombotic, antihypertensive and hypoglycaemic properties obtained from human and animal studies (Ardalani et al., 2016; Hajmohammadi et al., 2018; Hariri et al., 2020; Heydari et al., 2019; Sabzghabaee et al., 2014; Shidfar et al., 2014).

In the study, 81.3% of the participants reported that they had knowledge about the health benefits of sumac and 74.1% reported that they obtained this information from their family, spouse, friend, and relative. 20.9% of the participants stated that they consumed sumac every day. When the benefits of sumac were assessed based on individuals' own experiences, it was determined that sumac was effective for the treatment of stomach disorders (80.6%), the treatment of infection (79.9%), the treatment of influenza (74.1%), alleviating respiratory distress (67.6%), the treatment of gastric ulcer (63.3%), and lowering blood pressure (59.7%).

A meta-analysis reported that sumac may be beneficial in patients with metabolic syndrome and related disorders (Ghafouri et al., 2021). Likewise, it has been reported in the literature that daily intake of sumac may help in the treatment and/or prevention of metabolic syndrome and related diseases such as inflammation, cancer, and atherosclerosis (Khalil et al., 2021 b). The studies have revealed that sumac has positive effects on the lipid profile. (Hajmohammadi et al., 2018; Madihi et al., 2013; Rouhi-Boroujeni et al., 2016; Moon et al., 2015; Ehsani et al., 2022). However, in a meta-analysis, no definite conclusion could not be achieved about the effect of sumac on serum blood lipids (Akbari-Fakhrabadi et al., 2018).

The studies have reported that sumac consumption was effective in lowering blood glucose (Doğan and Çelik, 2016). In a meta-analysis, it was reported that sumac did not have a significant effect on various glycaemic indices such as fasting blood glucose level, HbA1c, insulin level and insulin resistance. However, as a result of the subgroup analysis, it was reported that there was a significant decrease in fasting blood glucose level (period ≥12 weeks and dose ≥2 g/day) and insulin resistance (BMI ≥30 kg/m2 and period <12 weeks) (Mohit et al., 2021).

In the study conducted by Gezici in 2019 it was found that sumac extracts inhibited the growth of lung cancer cells in a dose-dependent manner and stated that sumac fruits may have an important potential as an anti-lung cancer agent (Gezici, 2019). In another study, it was shown that sumac at 50 and 100 µM doses significantly inhibited the growth, proliferation, and viability of cancer cells (Gabr and Alghadir, 2021).

In a study, it was found that sumac fruit extract showed an antiviral activity against Herpes simplex virus 1 (Parsania et al., 2017). Another study revealed that the anti-neuroinflammatory activity of sumac included inhibition of the NF-kB signalling pathway (Khalil et al., 2021). All these study results support the sumac-related experiences of the individuals participating in the present study.

CONCLUSION

Consequently, it was found in the present study that the frequency of consuming sumac was 28.1% once a week and 20.9% every day. When the benefits of sumac were evaluated based on individuals' own experiences, it was observed that sumac was effective for the treatment of stomach disorders (80.6%), the treatment of infection (79.9%), the treatment of influenza (74.1%), alleviating respiratory distress (67.6%), the treatment of gastric ulcer (63.3%), and lowering blood pressure (59.7%).

It is recommended to conduct studies with larger samples and to increase the number of randomized controlled trials on this subject. It is important to use herbal approaches consciously. It is recommended to conduct larger studies on side effects and drug interactions that may occur especially in overdose.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Stomatal density, type and their relationships with leaf morphological traits in *Vitis vinifera* L. varieties

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Abstract
This study was carried out to determine the relationships between stomatal density and type and some leaf morphological traits in 10 grapevine varieties (*Vitis vinifera* L.). The study was performed during the vegetation period of 2022-2023 in the vineyards of the Research Station for Viticulture of Ankara University Faculty of Agriculture in Kalecik-Ankara. 'Lival', 'Tekirdağ Seedless', 'Beauty Seedless', 'Michele Palieri', 'Cabernet Sauvignon', 'Narince', 'Syrah', 'Kalecik Karası', 'Alphonse Lavallée' and 'Hasandede' varieties, grafted on 5BB rootstock, were evaluated as experimental materials in the study. The results revealed that there have been significant variations among varieties in terms of stomatal density, distribution of stomatal types, the proportion of trichomes, and leaf thickness. In general, stomatal density ranged from 168.17 to 268.27 stomata mm^{-2} in the varieties. The presence of the three different stomatal types (same level, raised above, and sunken) was detected by SEM images. The percentage of stomatal types varied between 39-59 for the same level, 25-42 for the raised above and 10-26 for the sunken. The percentage of the same level stomatal type was found to be higher than the other two types in all varieties. In addition, the same level stomatal type exhibited negative correlations with the raised above and the sunken stomatal types. In terms of leaf characteristics, the proportion of trichomes showed a negatively significant correlation ($r = -0.309$; $p<0.01$) with stomatal density. However, the relationship between leaf thickness and stomatal density was not significant.
Keywords: Grapevine, Stomatal density, Stomatal type, Sunken stomata, Correlations

INTRODUCTION

Stomata, located in the epidermis of plant leaves, serve as the starting point for metabolic processes, acting as the primary units for exchanging oxygen and carbon dioxide. They are essentially defined by two specialized guard cells in the leaf epidermis with a pore between them.

The presence and structure of stomata in the leaves of different plant species, even within a single species, show different morphological and anatomical characteristics. Stomata, along with their properties, are crucial structures for plants to respond to environmental conditions. Recently, new perspectives have been incorporated to study stomatal density, size and distribution patterns on leaves to analyse plant adaptation and evolution. There is a growing interest in using leaf and stomatal traits as rapid and reliable criteria to express the adaptability of plants to their ecological conditions (Franks et al., 2009; Sack and Buckley, 2016; Liu et al., 2018; Liu et al., 2021). On the other hand, the importance of generating information on leaf morpho-anatomical characters is emphasized

for evaluating the adaptability of genotypes against the possible consequences of climate change (IPCC 2013).

The measurable criteria for evaluating the adaptability of grapevines are mainly expressed in terms of developmental and yield characteristics. However, agricultural practices have an impact on the capacity of the vine, and therefore, the assessment of adaptation may be limited. The time-consuming criteria based on perennial grapevine development and yield contribute to the delay in recommending cultivars for ecology. However, there are not enough results on the functional effects of morpho-anatomical characteristics of leaves on the adaptation ability of grapevine. Recently, some approaches have been considered to understand the correlations with leaf traits (such as leaf shape, area, thickness, hairiness, cuticle, stomata, epidermis cell properties, and mesophyll anatomy) and stress conditions to develop alternative criteria for the assessment of adaptation (Boso et al., 2010; Samarth et al., 2021; MacMillan et al., 2021).

In particular, the characteristics of stomata in the species and the varieties of grapevine have been studied in relation to genotype, rootstock, and environmental conditions (Düzenli and Ağaoğlu, 1992; Shiraishi et al., 1996; Kara and Özeker, 1999; Marasalı and Aktekin, 2003; Gökbayrak et al., 2008; Rogiers et al., 2009; Keller, 2010; Hopper et al., 2014; İşçi et al., 2015; Boso et al., 2016; Uyak et al., 2016; Bodor et al., 2019; Doğan et al., 2020; Odabaşıoğlu, 2020; Candar et al., 2021; Copper et al., 2022). Currently, studies have been focused on the different stomatal types in grapevines, which have interested many plant species for decades. Thus, the role of stomata in the adaptability of genotypes in response to environmental conditions has been under investigation (Boso et al., 2011; Teixeira et al., 2018; Nassuth et al., 2021).

Studies on stomatal shape and the relationship between leaf morphological characteristics and stomata in grapevine species and cultivars are limited. Also, stomatal types of *Vitis vinifera* L. could not investigated in national viticultural literature. In this study, it was aimed to investigate the stomatal characteristics and their relations with some morphological traits of leaves in ten grapevine varieties of *Vitis vinifera* L. grown in Ankara-Kalecik conditions.

MATERIALS AND METHODS

The study was performed during the growing seasons of 2022-2023 on ten grapevine varieties cultivated in the vineyards of the Research Station for Viticulture of Ankara University Faculty of Agriculture in Kalecik-Ankara, Türkiye. The coordinates of the research area were 40°06′ 33.8′′ N 33°25′ 43.2′′ E, 670 m above sea level. The studied varieties were 'Lival', 'Tekirdağ Seedless', 'Beauty Seedless', 'Michele Palieri', 'Cabernet Sauvignon', 'Narince', 'Syrah', 'Kalecik Karası', 'Alphonse Lavallée', and 'Hasandede'. The grapevines were planted in 2005 and, after being grafted on 5 BB rootstocks, were spaced 1.5x3 m rows and were trained at a double cordon having 80 cm stem height. Drip irrigation was applied 4-5 times from bud-burst to pre-véraison. The vineyard soil was clay-loam with a pH of 7.5. The research region has a continental climate. The experimental design consisted of three replications and randomly selected five vines for each replication per variety.

Collecting of leaf samples

Five leaf samples were hand-collected from the vines of each replication for stomatal and leaf morphological examinations. According to the OIV (2001) definition of mature leaves, the collection was performed between the berry set and the véraison period. The sampling was performed on July 18, 2022 and July 22, 2023. To ensure uniformity, leaves were collected from the 8th and 9th nodes in the same direction across all vines. To avoid dehydration and discolouring, leaf samples were transported in cooler bags and were transferred to the Cytology Laboratory of the Department of Horticulture, Ankara University.

Determination of stomatal density

The leaf print removal method was used to determine stomatal density in fresh leaf samples. Leaf prints were removed from the lower surface of the leaf blade using nail polish. Leaf prints were obtained from a total of 6 areas on a leaf blade, including around the petiole sinus, both sides of the main vein and lateral veins. These prints were prepared for examination using a light microscope (LM-Zeiss Axiolab). During the LM examination of the slides, microphotographs were taken to create the digital archive for further measurements. In microscopic studies, mainly 40x magnification was used. Stomatal density (stomata mm^{-2}) was determined in microphotograph areas of 0.037 mm^2.

Determination of stomatal type

The stomatal type was determined through LM and SEM (Scanning Electron Microscope) examinations. SEM studies were performed on well-dried leaf samples. The gold-coated process was provided by the supervision of the Science Application and Research Center of Van Yuzuncu Yıl University. The definitions were realized on the SEM microphotographs according to Monteiro et al. (2013), Teixeira et al. (2018), and Šantrůček et al. (2022).

Examination of leaf morphological traits

In the present study, leaf area (cm^2), the proportion of trichomes (%) and leaf thickness (mm) were examined.

Leaf areas were calculated from fresh leaf images, trichomes were examined using a stereo microscope (Olympus SZ40), and microphotographs were taken of the abaxial surfaces of fresh leaves. Here, it was preferred to use the term trichome as a collective term for two different hair structures, consisting of both prostrate and erect type hairs, rather than hairiness. Computations on all digitised observations were analysed using the ImageJ/IJ 1.46r program (https://imagej.nih.gov/ij/index.html). In addition to the digitised-based calculations, the OIV (2001) scale was used to score the density of the prostrate and erect hairs. Leaf thickness was measured using a digital micrometer device (INSIZE 3109-25A), and thickness values were grouped according to Bozkurt (2023).

Statistical analysis

Descriptive statistics for the continuous variables were presented as Mean and Standard error of mean. One-way ANOVA was used to compare group means. Following the ANOVA, Duncan multiple comparison test was performed to identify different varieties' means. Pearson correlation coefficients were computed to determine linear relationhips between the variables. In addition to correlation analysis, Linear regression analysis was performed to predict dependent variables with independent variables. Statistical significance level was considered as 5% and 1%. SPSS (ver: 26) statistical program was used for all statistical computations.

RESULTS AND DISCUSSION

Stomatal density (stomata mm^{-2})

Stomatal density varied significantly among all grapevine varieties, ranging from 168.2 to 268.3 stomata per mm^2. The varieties with the highest stomatal density were 'Lival' (268.3 stomata mm^{-2}), 'Tekirdağ Seedless' (265.3 stomata mm^{-2}), and 'Beauty Seedless' (258.3 stomata mm^{-2}). 'Hasandede' showed the lowest stomatal density, with 168.2 stomata per mm^2 (Figure 1). Many researchers reported that stomatal densities varied significantly among grapevine varieties. Keller (2010) stated a wide range of 50-400 stomata mm^{-2} for *Vitis* species. Previous studies investigating stomatal density under the influence of genotype and environmental conditions reported the following findings: Shiraishi et al. (1996), 136.1-302.6 stomata mm^{-2}; Kara and Özeker (1999), 208.3- 294.8 stomata mm^{-2}; Marasalı and Aktekin (2003), 156.1-269.5 stomata mm^{-2}; Gökbayrak et al. (2008), 190.9-220.6 stomata mm^{-2}; Rogiers et al. (2009), 162.0-232.6 stomata mm^{-2}; Monteiro et al. (2013), 206.7-285.7 stomata mm^{-2}; Bekişli (2014), 150.9-189.3 stomata mm^{-2}; İşçi et al. (2015), 67.2-188.9 stomata mm^{-2}; Monteiro et al. (2018), 179-256 stomata mm^{-2}; Teixeira et al. (2018), 170-250 stomata mm^{-2}; Bodor et al. (2019), 94.8-157.0 stomata mm^{-2}; Nassuth et al. (2021), 178.0-354.5 stomata mm^{-2} and Copper et al. (2022), 139.8-238.6 stomata mm^{-2}. Apart from genotype and environmental factors, variations in stomatal density were attributed to microscopic miscounting. Therefore, LM and SEM examinations were evaluated together in this study to prevent such errors. Figure 2 shows the image clarity for stomatal evaluation in this study.

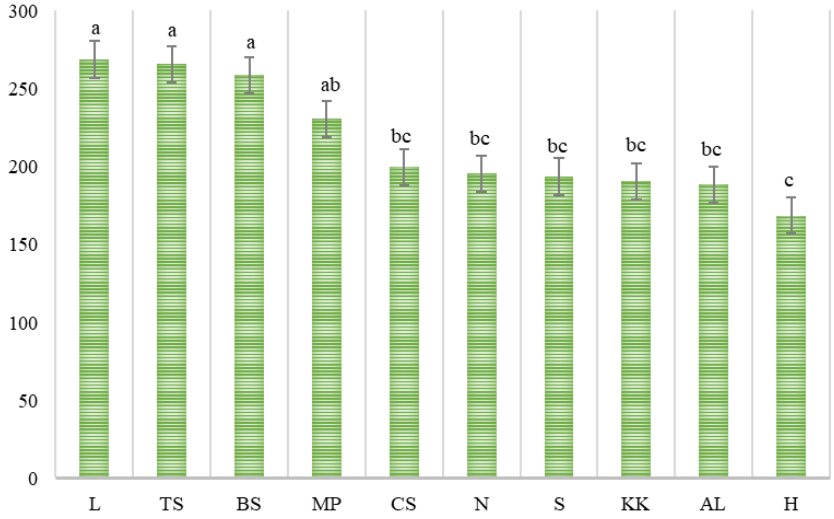

Figure 1. Stomatal density of grapevine varieties (stomata mm^{-2}). Lival-L; Tekirdağ Seedless-TS; Beauty Seedless-BS; Michele Palieri- MP; Cabernet Sauvignon-CS; Narince-N; Syrah-S; Kalecik Karası-KK; Alphonse Lavallée-AL; Hasandede-H (Different lower cases represent statistically significant differences among the varieties).

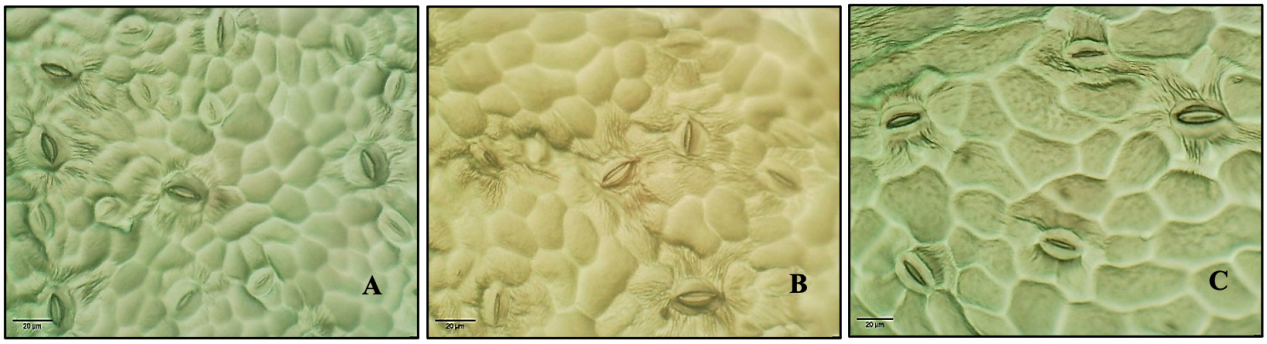

Figure 2. Images of stomata with different densities under a light microscope. A-'Beauty Seedless'; B-'Kalecik Karası'; C- 'Hasandede' (Scale bar=20 µm).

Stomatal types and distribution in grapevine varieties

Three different stomatal types reported for *Vitis* species were detected in the leaves of ten grapevine varieties. These types were classified as (1) same level, (2) sunken and (3) raised above, depending on the structural height of the guard cells on the leaf surface relative to subsidiary cells (Swanepoel and Villers, 1987; Monteiro et al., 2013; Teixeira et al., 2018). SEM images used to determine the stomatal types were given in Figures 3, 4 and 5. The proportion of the same level stomata varied between 39.12% (Syrah) and 59.41% (Alphonse Lavallée) in evaluating the distribution of stomatal types in the varieties. The proportion of the raised-above stomata ranged from 25.39% (Alphonse Lavallée) to 42.63% (Hasandede), while the proportion of the sunken stomata ranged from 10.53% (Beauty Seedless) to 26.47% (Cabernet Sauvignon) (Figure 6). Based on the visual representation in Figure 6 and the statistical interpretation of the results in Table 1, it was determined that the distribution of stomatal types within the same cultivar was significant. The percentage of the same level stomata in all varieties was higher than the other two types. Hasandede was the only variety that deviated from this rule. There was a minimal and statistically insignificant difference between the same level and the raised above stomata in this variety. In addition, the sunken stomata showed the lowest percentage of all varieties. Monteiro et al. (2018) emphasized that stomatal types differ among varieties. In this study, it was observed that the sunken stomatal type was smaller than the other two types. This type was characterized by guard cells buried between the subsidiary cells. Monteiro et al. (2018) and Teixeira et al. (2018) emphasized that genotypes with high stomatal density and the sunken stomata may be more advantageous against abiotic stress conditions, especially in hot and arid ecologies. Furthermore, Nassuth et al. (2021) reported that sunken stomata can also be advantageous at low temperatures and are present in cold-tolerant varieties. Jones (2014), Serra et al. (2017), and Teixeira et al. (2018) stated that high stomatal density and sunken stomata are important in reducing plant transpiration and that these traits play a role in the adaptation of varieties to water stress.

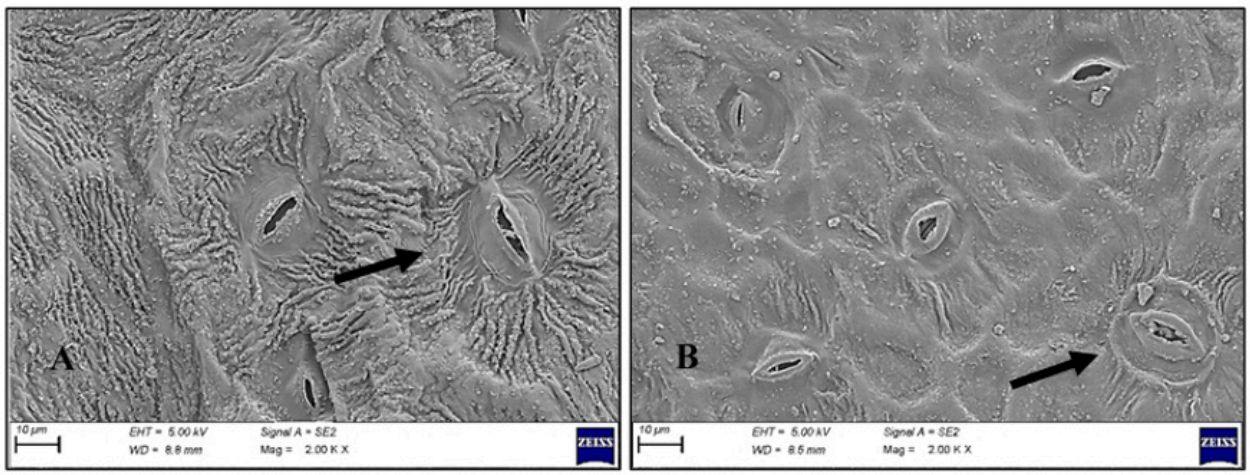

Figure 3. SEM image of the raised above stomata. A-'Narince'; B-'Tekirdağ Seedless'

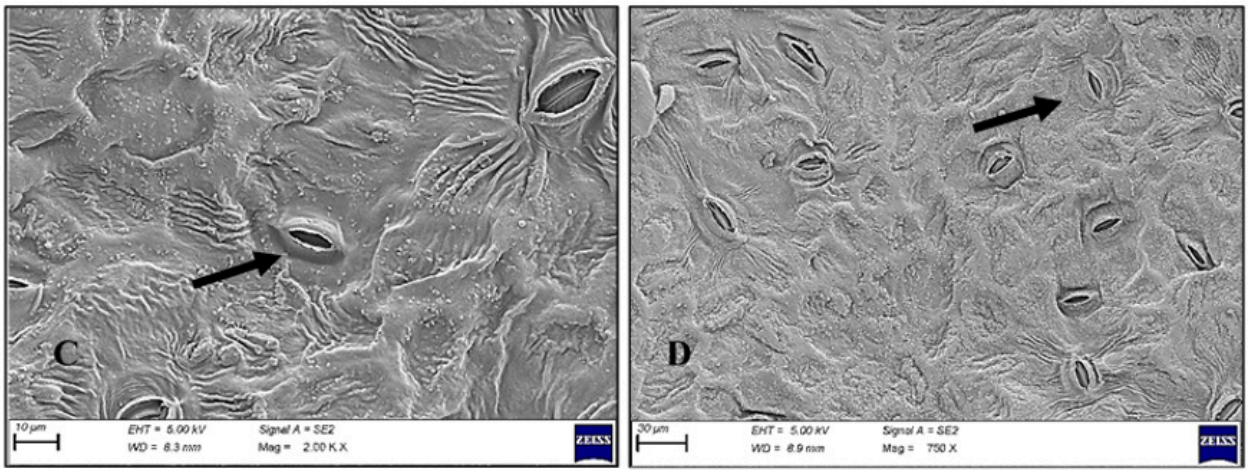

Figure 4. SEM image of the same level stomata. C-'Michele Palieri'; D-'Cabernet Sauvignon'

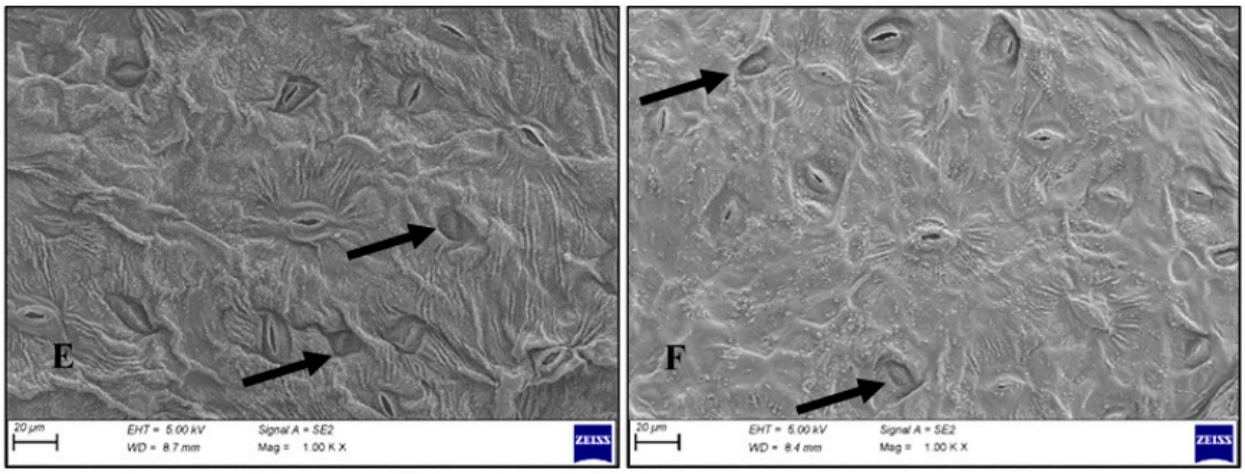

Figure 5. SEM image of the sunken stomata. E-'Alphonse Lavallee'; F-'Beauty Seedless'

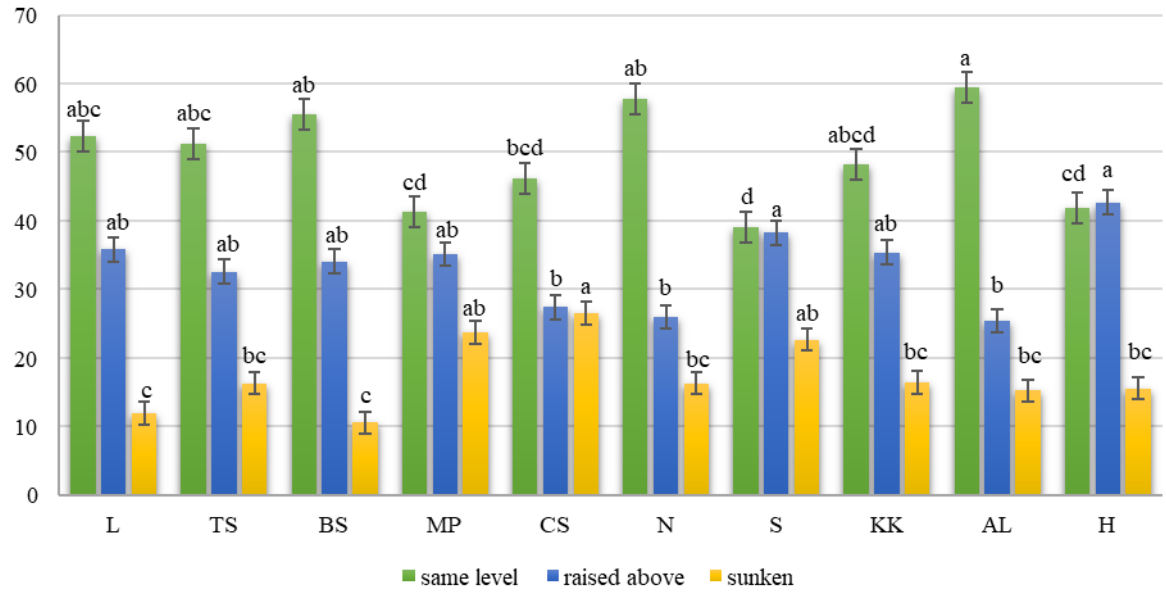

Figure 6. The distribution of stomatal types among grapevine varieties (%). Lival-L; Tekirdağ Seedless-TS; Beauty Seedless-BS; Michele Palieri- MP; Cabernet Sauvignon-CS; Narince-N; Syrah-S; Kalecik Karası-KK; Alphonse Lavallée-AL; Hasandede-H (Different lower cases represent statistically significant differences among the varieties).

Table 1. The distribution of stomatal types in grapevine varieties (%)

Variety	Stomatal Type		
	Same level (Mean ± SEM)	Raised above (Mean ± SEM)	Sunken (Mean ± SEM)
Lival	52.28 ± 3.68 a	35.82 ± 3.17 b	11.90 ± 2.70 c
Tekirdağ Seedless	51.13 ± 1.38 a	32.57 ± 2.03 b	16.29 ± 3.20 c
Beauty Seedless	55.45 ± 5.23 a	34.02 ± 2.14 b	10.53 ± 3.35 c
Michele Palieri	41.21 ± 3.18 a	35.10 ± 4.32 ab	23.69 ± 3.84 c
Cabernet Sauvignon	46.18 ± 2.35 a	27.36 ± 0.93 b	26.47 ± 2.43 b
Narince	57.79 ± 3.98 a	25.93 ± 1.70 b	16.29 ± 2.28 b
Syrah	39.12 ± 3.28 a	38.25 ± 3.99 a	22.63± 0.85 b
Kalecik Karası	48.26 ± 3.99 a	35.33 ± 5.12 a	16.41 ± 2.04 b
Alphonse Lavallée	59.41 ± 4.09 a	25.39 ± 3.58 b	15.20 ± 1.55 b
Hasandede	41.84 ± 4.32 a	42.63 ± 3.06 a	15.54 ± 1.87 b

* Different lowercase letters on the same line indicate significant differences of stomatal types in each variety ($p<0.05$). SEM: Standard error of Mean

Leaf morphological traits and stomatal relationships

The average size of the leaves collected during the period from berry set to veraison in Kalecik conditions is shown in Table 2. The leaf area ranged from 72.21 cm^2 (Syrah) to 95.09 cm^2 (Tekirdağ Seedless), with no statistically significant difference. This result allowed us to analyse stomatal density on comparable leaf areas. Bekişli (2014) found the relationship between leaf area and stomatal density at a value of $R^2 = 0.0498$. Thus, the correlation between leaf area and stomatal density was not statistically significant. Similarly, Boso et al. (2016) reported that this relationship was not significant. The study investigated the relationships between the proportion of trichomes and leaf thickness, stomatal density, and the distribution of density by stomatal type. The results are presented in Table 5 and Figure 7. The proportion of trichomes (%) was found to be significant among the varieties. 'Kalecik Karası' exhibited the highest percentage at 39.75%, followed by 'Syrah' at 16.29% and 'Alphonse Lavallée' at 14.91%. 'Beauty Seedless', 'Michele Palieri', and 'Tekirdağ Seedless' exhibited the lowest percentages (Table 2). In addition, the density of prostate and erect hairs between the main veins on the lower side of blade in the varieties was classified according to the OIV 84 and OIV 85 codes (Table 3). The trichome percentage values (the sum of prostrate and erect hairs) of the varieties were in line with the scoring results obtained according to OIV (2001). Monteiro et al. (2018) and Gago et al. (2019) reported differences in leaf anatomical tissue thickness among grapevine varieties. Additionally, Gago et al. (2016) found variations in spatial density and relative abundance of trichomes among grapevine varieties.

There was a significant difference in leaf thickness among the varieties. Regarding the leaf thickness, higher values were found in 'Kalecik Karası' (0.73 mm), 'Narince' (0.63 mm) and 'Michele Palieri' (0.61 mm). The lowest thickness value (0.45 mm) was found for 'Tekirdağ Seedless' (Table 2). According to the method described by Bozkurt (2023), 'Kalecik Karası' was placed in the thick group, while 'Narince', 'Beauty Seedless', 'Michele Palieri', and 'Hasandede' were placed in the medium group. 'Alphonse Lavallée', 'Lival', 'Cabernet Sauvignon' and 'Syrah' were classified in the thin group, and 'Tekirdağ Seedless' was placed in the very thin group (Table 4).

The correlation analysis showed that there was a significant negative correlation ($r = -0.309$; $p<0.01$) between the stomatal density and the proportion of trichomes (%). However, there was no significant relationship with leaf thickness. A significant positive correlation was found between the proportion of trichomes (%) and leaf thickness (mm) ($r = 0.528$; $p<0.01$) (Table 5). It was possible to interpret from this study that trichome density was generally lower or absent in the varieties with a high stomatal density. Boso et al. (2011) found similar results and emphasized the importance of the relationship between stomatal density and trichome density. 'Kalecik Karası' showed the highest values in the proportion of trichomes (39.75%) and leaf thickness. This result confirmed the relationship obtained.

In the evaluation of the relationships by the stomatal types, negative correlations were found between the same level stomata and the raised above stomata ($r = -0.704$, $p<0.01$), as well as between the sunken stomata and the same level stomata ($r = -0.574$, $p<0.05$). However, no such association have been reported for stomatal types in previous studies.

Table 2. Leaf morphological traits in grapevine varieties

Variety	Leaf area (cm^2) (Mean ± SEM)	Proportion of trichomes (%) (Mean ± SEM)	Leaf thickness (mm) (Mean ± SEM)
Lival	87.73 ± 5.30	14.04 ± 2.85 bc	0.54 ± 0.02 cd
Tekirdağ Seedless	95.09 ± 4.85	0.41 ± 0.12 e	0.45 ± 0.00 e
Beauty Seedless	91.06 ± 8.81	0.00 ± 0.00 e	0.57 ± 0.01 bc
Michele Palieri	92.81 ± 3.03	0.00 ± 0.00 e	0.60 ± 0.03 bc
Cabernet Sauvignon	90.86 ± 8.87	7.42 ± 0.21 d	0.51 ± 0.14 d
Narince	83.21 ± 2.55	11.03 ± 0.28 cd	0.63 ± 0.03 b
Syrah	72.21 ± 0.84	16.29 ± 1.14 b	0.51 ± 0.14 d
Kalecik Karası	82.43 ± 0.04	39.75 ± 2.80 a	0.73 ± 0.03 a
Alphonse Lavallée	85.19 ± 3.21	14.91 ± 1.13 bc	0.48 ± 0.02 de
Hasandede	75.91 ± 2.52	1.98 ± 0.93 e	0.58 ± 0.01 bc

*Different lowercase letters in the same column indicate significant differences among varieties for each trait ($p<0.05$). SEM: Standard error of Mean

Table 3. Density of prostrate and erect hairs between main veins on the lower side of the blade: 1 = none or very low, 3 = low, 5 = medium, 7 = high, 9 = very high (OIV 2001)

Variety	Density of prostrate hairs (OIV 84)	Density of erect hairs (OIV 85)
Lival	3	3
Tekirdağ Seedless	1	1
Beauty Seedless	1	1
Michele Palieri	1	1
Cabernet Sauvignon	3	3
Narince	3	3
Syrah	3	3
Kalecik Karası	5	5
Alphonse Lavallée	3	3
Hasandede	1	5

Table 4. Range values for the average leaf thickness class of the varieties (mm)

Very thin	Thin	Medium	Thick	Very thick
0.356-0.448	0.449-0.541	0.542-0.634	0.635-0.727	0.728-0.821
Tekirdağ Seedless	Alphonse Lavallée Lival Cabernet Sauvignon Syrah	Narince Beauty Seedless Michele Palieri Hasandede	Kalecik Karası	

Table 5. Correlation analysis between stomatal density, type and leaf morphological traits

		Stomatal density (stomata mm^{-2})	Proportion of trichomes (%)	Leaf thickness (mm)	Stomatal type		
					Raised above	Same level	Sunken
Stomatal density (stomata mm^{-2})		1					
Proportion of trichomes (%)		-0.309*	1				
Leaf thickness (mm)		-0.284	0.528**	1			
Stomatal type	Raised above	-0.074	-0.041	0.124	1		
	Same level	0.154	0.081	0.016	-0.704**	1	
	Sunken	-0.128	-0.065	-0.165	0.174	-0.574*	1

*: $p<0.05$ **: $p<0.01$

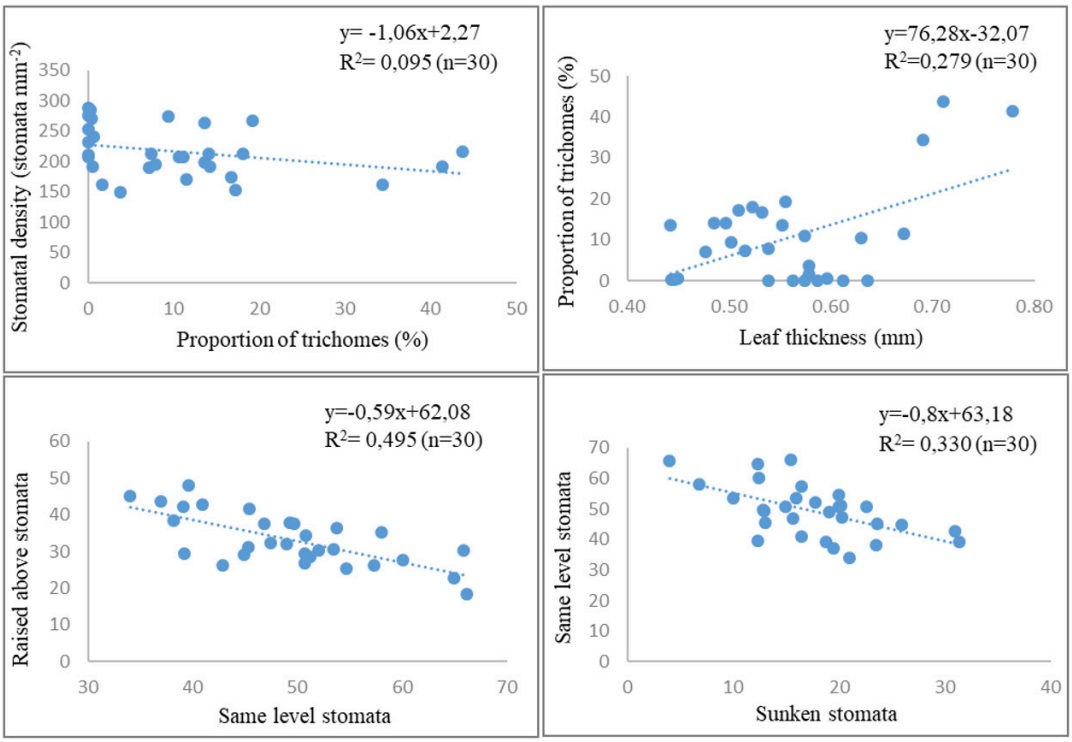

Figure 7. Regression graphs for stomatal density, type and leaf morphological traits.

CONCLUSION

The study revealed stomatal density was a cultivar-specific characteristics for grapevine varieties (*Vitis vinifera* L.). This result is consistent with previous findings that grapevines grown in different ecological conditions. It is thought that, variations in stomatal density among grapevine varieties were probably caused by the intrinsic characteristics of the cultivars in relation to the conditions of their growing ecologies. The relationship between stomatal characteristics and adaptation ability of varieties is discussed. One of the aims of this study is to draw attention to this argument. It was thought that the percentage of sunken stomatal types could be used to evaluate the adaptation of varieties to the semi-arid conditions of Kalecik. In addition, correlation analysis revealed interest in trichome proportion, leaf thickness and stomatal characteristics. To improve vineyard management, it is necessary to gather more information on the impact of climate change on viticulture. Research that examines stomatal and leaf characteristics can provide a new approach to adaptation studies. Finally, the study is the first report on stomatal types in the viticultural literature of Türkiye.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

All authors declare that they have no conflict of interest.

Author contribution

Sinem Güler and Birhan Kunter were responsible for the experimental design, data acquisition and analysis, and manuscript drafting. Aysun Şehit contributed to data analysis. All authors read and approved the final version of the manuscript.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication
Not applicable.
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RESEARCH ARTICLE

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Molecular characterization of Turkish hazelnut cultivars and genotypes using SSR markers

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Abstract
This study defines the genetic characterization of 16 hazelnut varieties and 64 genotypes. SSR method was used in molecular characterization studies. 18 SSR primers were used. In the SSR method, 118 bands were obtained, and 115 were polymorphic. The similarity coefficient in overall genotypes were between 0.12 and 0.98 in SSR a n d the polymorphism rate of the primer pairs used was calculated as 98.0%. The lowest polymorphism information content value (0.542) was obtained from the CAC- C 028 locus, and the highest polymorphism information content value (0.987) was obtained from the CAC- B 113 locus. The average polymorphism information content value was 0.786. The total discrimination power values of SSR loci were determined as 17.98; the lowest (0.748) discrimination power value was obtained from the CAC- B 020 locus, while the highest (1.404) discrimination power value was obtained from the CAC- A 024 b locus. The genotypes'clustering positions across the dendograms were essentially correlated with their geographic origins. This result shows that genetic origin plays an important role in terms of classification of genotypes in hazelnut.

Keywords: Hazelnut, molecular characterization, SSR

INTRODUCTION

Hazelnuts belong to the genus *Corylus* of the subfamily Coryleae of the family Betulaceae of the order Fagales in the systematics and grow in temperate regions of the northern hemisphere. *Corylus* species are diploid and have a chromosome number of 2n = 2x = 22. More than 25 species have been described within the genus Corylus. Among these species, *C. avellana*, *C. colurna* and *C. maxima* Mill are commercially important (Özbek, 1978). Turkish hazelnut varieties are hybrids of *C. avellana* and *C. maxima* (Okay et al., 1986). It has been reported that Central Asia, Caucasus, and Anatolia are the homelands of hazelnut, and hazelnut was first cultivated in Anatolia in the Eastern Black Sea Region (Islam, 2000). Hazelnut plantations are found in the Black Sea and Marmara Regions of Turkey, in Tokat, Adana, and Mersin provinces, and in the Vangölü Basin, which includes Bitlis province (Islam and Özgüven, 1997). Turkey is among the richest gene sources of cultivated hazelnuts and has rich genetic material due to thousands of years of cultivation culture. Wild hazelnuts, which are formed as a result of natural hybridizations and are still found in the gardens of producers, increase this variation even more. Turkey has natural populations that are very important in breeding among hazelnut-growing countries. In Turkey, 20 standard hazelnut cultivars have been identified as 'Tombul', 'Palaz', 'Çakıldak', 'Foşa', 'Mincane', 'Uzunmusa', 'Cavcava', 'Kargalak', 'Kan', 'Kalınkara', 'İncekara', 'Sivri', 'Karafındık', 'Yassı Badem' and 'Yuvarlak Badem', Girusun Melezi, Okay 28, Allahverdi, Yomrali and Çetiner (Ayfer et al., 1986; Köksal, 2002; HRI,2024). It is important that the genetic material used in plant breeding and variety development studies is correct to its name and that the genetic relationships between them are determined. To obtain

patent rights for new varieties and to have the sanctioning power of the laws, methods that can distinguish varieties from each other precisely should be used. Developments in molecular markers provide great convenience in this regard. Investigation of hazelnut gene resources using molecular markers, identification of species and cultivars, and studying of inheritance markers provide the emergence of agriculturally important characters. SSR or microsatellites consist of 2-6 nucleotide groups distributed throughout eukaryotic genomes and repeated consecutively. The DNA sequences surrounding microsatellites are usually conserved among individuals of the same species, allowing the selection of overlapping SSRs in different genotypes by amplification with PCR primers. The difference in the number of consecutive SSR repeats results in PCR amplification of fragments of different lengths. These repeats are highly polymorphic due to mutations that lead to changes in the number of repeated units, even for very close species and varieties. Different alleles at a locus can be detected by PCR using the conserved DNA sequences surrounding SSRs as primers. Since SSRs are highly polymorphic, they are highly informative in plants. SSRs are very abundant in plant genomes and have a uniform distribution. Due to these advantages, using the SSR technique in genetic mapping studies in plants is increasing daily (Özcan et al., 2001). In this study, it was aimed to identify the molecular characteristics of hazelnut varieties cultivated in Turkey, clones of some important varieties, and hazelnut types cultivated in the Vangölü basin by the SSR marker method.

MATERIALS AND METHODS

Plant materials

In this study, Turkish hazelnut varieties in the genetic resources orchard of Giresun Hazelnut Research Institute, Tombul, Palaz, Foşa, Çakıldak, Mincane, Cavcava, Uzunmusa, Sivri, Yassı Badem, Yuvarlak Badem, Kargalak, Kalınkara, İncekara, Kuş, Acı, Kan, varieties and 45 hazelnut genotypes in the same institute, which were determined by selection studies carried out in previous years, and in addition to these, 17 genotypes selected from the Vangölü basin and 2 genotypes taken from Çorum province were used. This study was conducted between 2005-2008. In addition, DNAs of 4 types of *C. colurna* species in Giresun Hazelnut Research Institute were also used. The tag numbers of the genotypes taken from the genetic resources parcel of Giresun Hazelnut Research Institute were used exactly based on the numbers in the institute records. SSR analyses were conducted in the Biotechnology laboratory of Çukurova University, Faculty of Agriculture, Department of Horticulture.

Microsatellite (SSR) Analyzes

Newly blooming young leaves of the cultivars were used for DNA isolation. The leaf samples were washed with 50% alcohol, dried, and placed in liquid nitrogen (-196 ºC), and kept at -80 ºC until DNA isolation. The "minipreparation" DNA isolation method was modified by Doyle and Doyle (1990). The primers used in SSR analysis were selected by Mehlenbacher et al. (2006) by considering the primers used for hazelnuts in different studies which gave positive results in these studies. The 18 SSR primers (Operon Technologies) were labeled with a fluorescent label for use in the Li-Cor imaging system. SSR analyses were performed according to the method modified by Kaçar (2001). The total volume was adjusted to 20 µl for each sample and consisted of 8 µl 2XPCR Master Mix (Fermantas), 1 µl Primer (forward + reverse), 0.5 µl $MgCl_2$ (25 mM), 0.5 µl M13 primer, 0.05 µl Taq DNA Polymerase, 5 µl ddH_2O, 5 µl genomic DNA (5ng). PCR cycling conditions were applied, as shown in Table 1.

Table 1. PCR Cycle Conditions in SSR Analysis

Process	Temperature (ºC)	Duration	Cycle
Pre-Denaturation	94ºC	5 min	1
Denaturation	94ºC	1 min	
Annealing	55-60ºC	30 sn	35
Elongation	72ºC	1 min	
Final elongation	72ºC	4 min	1

Polymorphism rates of the primers were calculated by dividing the number of polymorphic bands by the total number of bands and multiplying by 100. The polymorphism information content (PII) of the SSR primers used was determined using the formula "PII = 1 - Σ Pi2" according to Smith et al. (1997). According to this method, the total number of present (1) and absent (0) bands in polymorphic bands was determined and frequency values were calculated for each of these bands (Pi: frequency of band i). The discrimination power of SSR primers was calculated according to the formula developed by Prevost and Wilkinson (1999); discrimination power = Σ Ib (Ib = 1 - (2 X $\left|0.5 - p\right|$)). p in the formula is the ratio of I band in the total genotype.

The PCR products obtained were electrophoresed in polyacrylamide gel using a Li-Cor imaging system, and band images were obtained. For this purpose, 6.5% polyacrylamide gel was prepared. The amplification products obtained from PCR cycles were separated by agarose gel electrophoresis. The bands formed were evaluated as present (1) or

absent (0). The numerical information obtained was analyzed using the computer program NTSYSpc 2.0V (Numerical Taxonomy and Multivarious Analysis System, Version 2.0V) (Rohlf, 2004). The genetic similarity index between genotypes was calculated using the Jaccard coefficient, and pedigree analysis was obtained using the UPGMA method.

RESULTS AND DISCUSSION

Total allele numbers, polymorphic allele numbers, polymorphism rate, polymorphism information content and discrimination power values obtained as a result of PCR and electrophoresis applications are presented in Table 2. A total of 118 alleles were obtained from 18 SSR loci, and 115 were polymorphic. The total number of alleles per primer varied between 2 and 11 (mean 6.55). The number of polymorphic bands per locus varied between 2 and 11 (mean 6.38). The differences in the number of alleles are due to the different genotypes used in the study. Regarding the total number of alleles, the CAC- B 110 locus produced the least number of alleles (2), and the CAC- A 014 b locus produced the highest number of alleles (11). The polymorphism rate of the primer pairs used was determined as 98.0%. The lowest (0.542) polymorphism information content value was obtained from CAC- C 028 locus and the highest (0.987) polymorphism information content value was obtained from CAC- B 113 locus. The average polymorphism information content value was 0.786. Total discrimination power values of SSR loci were determined as 17.98; the lowest (0.748) discrimination power value was obtained from CAC- B 020 locus, whereas the highest (1.404) discrimination power value was obtained from CAC- A 024 b locus. The gel image obtained as a result of PCR amplification of CAC- B 109 primer with 84 hazelnut genotypes is presented in Figure 1. In this image, 9 out of 10 alleles were polymorphic. Bassil et al. (2003) tested 12 SSR primers in a study with 19 hazelnut genotypes and determined that the number of alleles per microsatellite locus varied between 4 and 7 and the heterozygosity ratio varied between 0.58 and 0.87. Boccacci et al. (2006), using 78 hazelnut cultivars and 16 SSR loci, found that the number of alleles per locus was 9.4, with high allele frequencies up to 78% and a discrimination power of 91%. Gökırmak et al. (2008) used 21 SSR primers to analyze the genetic profiles of 270 clones of *C. avellena* species. It was reported that the average number of alleles per locus was 9.81 and heterozygosity rate was 0.67 in 198 clones showing different genetic profiles. In a study conducted by Yang et al. (2021) in China, 27 SSR primers were used to identify 57 Ping'ou hazelnut hybrids. The results showed heterozygosity (Ho) of 0.84, expected heterozygosity (He) of 0.80, and polymorphism information content (PIC) of 0.78. A total of 301 alleles were detected and the number of effective alleles ranged from 11.2 to 18 alleles per locus on average. Polymorphism rates of the loci used in the study were higher than in previous studies. The presence of *C. avellana* and *C. colurna* species among the genotypes used in the study can be considered among the reasons for the high polymorphism rates.

Table 2. Total band numbers, polymorphic band numbers, polymorphism rate, polymorphism information content and discrimination power values obtained from SSR primers

No	Primer	Total band	Polymorphic band	Polymorphism rate (%)	Polymorphism information content	Discrimination power values
1	CAC- A 014 a	10	9	90	0.742	1.154
2	CAC- A 014 b	11	11	100	0.724	0.845
3	CAC- A 024 b	5	5	100	0.671	1.404
4	CAC- A 36	5	5	100	0.784	0.822
5	CAC- A 105	7	6	86	0.741	1.451
6	CAC- B 001	6	6	100	0.776	1.282
7	CAC- B 020	8	8	100	0.674	0.748
8	CAC- B 028	7	7	100	0.747	0.815
9	CAC- B 029 b	10	10	100	0.771	0.774
10	CAC- B 109	10	9	90	0.582	0.825
11	CAC- B 110	2	2	100	0.728	0.852
12	CAC- B 111	5	5	100	0.787	0.958
13	CAC- B 113	8	8	100	0.987	0.752
14	CAC- B 114	5	5	100	0.784	0.958
15	CAC- C 003	5	5	100	0.775	1.225
16	CAC- C 111	2	2	100	0.672	0.958
17	CAC- C 118	6	6	100	0.714	1.242
18	CAC- C 028	6	6	100	0.542	0.817
	Total	118	115	--	--	17.98
	Average	6.55	6.38	98	0.786	

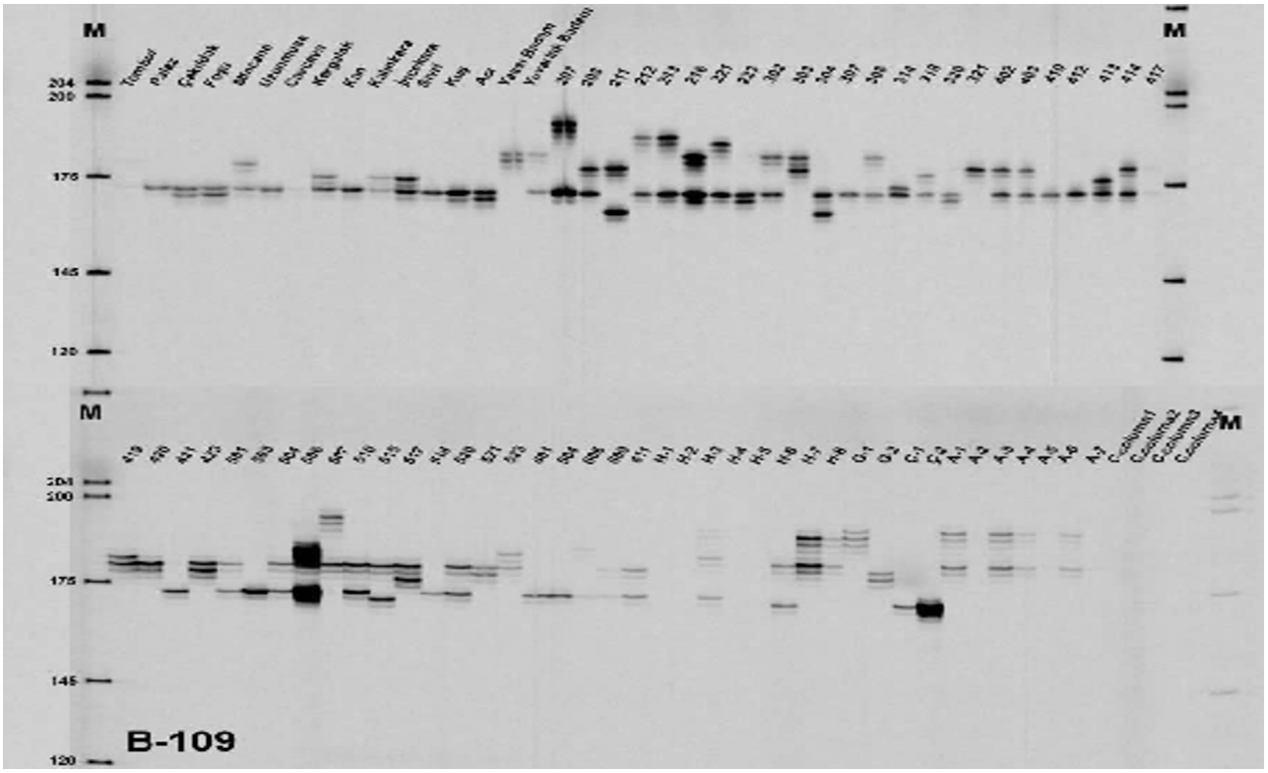

Figure 1. Vertical electrophoresis gel image of SSR primer CAC-B 109

In the dendrogram created as a result of SSR analyses, the similarity ratio for all genotypes ranged between 0.250 and 0.983. This dendrogram obtained on the basis of SSR analyses was first divided into two main branches numbered 1 and 2 (Figure 2). In the main branch numbered 1, four genotypes belonging to *C. colurna* species were included. *C. colurna* species were found to be 25% close to and approximately 75% distant from all other genotypes used in the study. *C. colurna* types formed two branches among themselves and the similarity ratio between these branches was 0.580. According to SSR results, this species, which has pomologically small fruits and different morphological characteristics, also differed genetically from the other hazelnut genotypes in the study. The main branch numbered as 2 on the dendrogram was again divided into 2 subgroups. These two subgroups were numbered 2.1 and 2.2 on the dendrogram. These two subgroups were genetically close to each other by about 28%. Genotypes Ç1 and Ç2 were included in the subgroup numbered 2.1. According to the SSR results, the similarity index between the genotypes Ç1 and Ç2 taken from Çorum province within the scope of the experiment and the *C. colurna* genotypes examined in the SSR analysis was determined as 0.250. The similarity index of Ç1 and Ç2 genotypes with genotypes other than *C. colurna* types was 0.280. The subgroup numbered 2.2 on the dendrogram was divided into two branches. These two branches are numbered 2.2.1 and 2.2.2 on the dendrogram. These two branches were found to be 42% close to each other. Among the hazelnut genotypes taken from Vangölü Basin, all genotypes except G-2 were collected in the branch numbered 2.2.1 on the dendrogram. It is noteworthy that the Cavcava variety is also located in this branch. Within the scope of the study, 15 standard varieties and 46 genotypes were clustered in the group numbered 2.2.2. Fiore et al. (2022), in their study conducted with 9 SSR primers in 75 hazelnut genotypes selected from the Sicily region.Authors reported that they distinguished between local genotypes and commercial varieties and that the Nebrodi genotypes examined showed significant differences from Northern Italian, Iberian, and Turkish genotypes.

CONCLUSION

Standard Turkish hazelnut varieties, genotypes from the genetic resources parcel of Giresun Hazelnut Research Institute, and hazelnut genotypes from Vangölü Basin were separated from each other on the pedigree tree obtained by molecular methods. This result shows that genetic origin plays an important role in the classification of genotypes. It has been reported that in the Black Sea region where hazelnut is intensively cultivated in our country, our important standard varieties have synonyms. This is an important source of confusion for researchers in a variety of identification studies. Using the primer and molecular techniques used in this study, similarity, and dissimilarity rates between the varieties and genotypes reported to be synonymous can be determined.

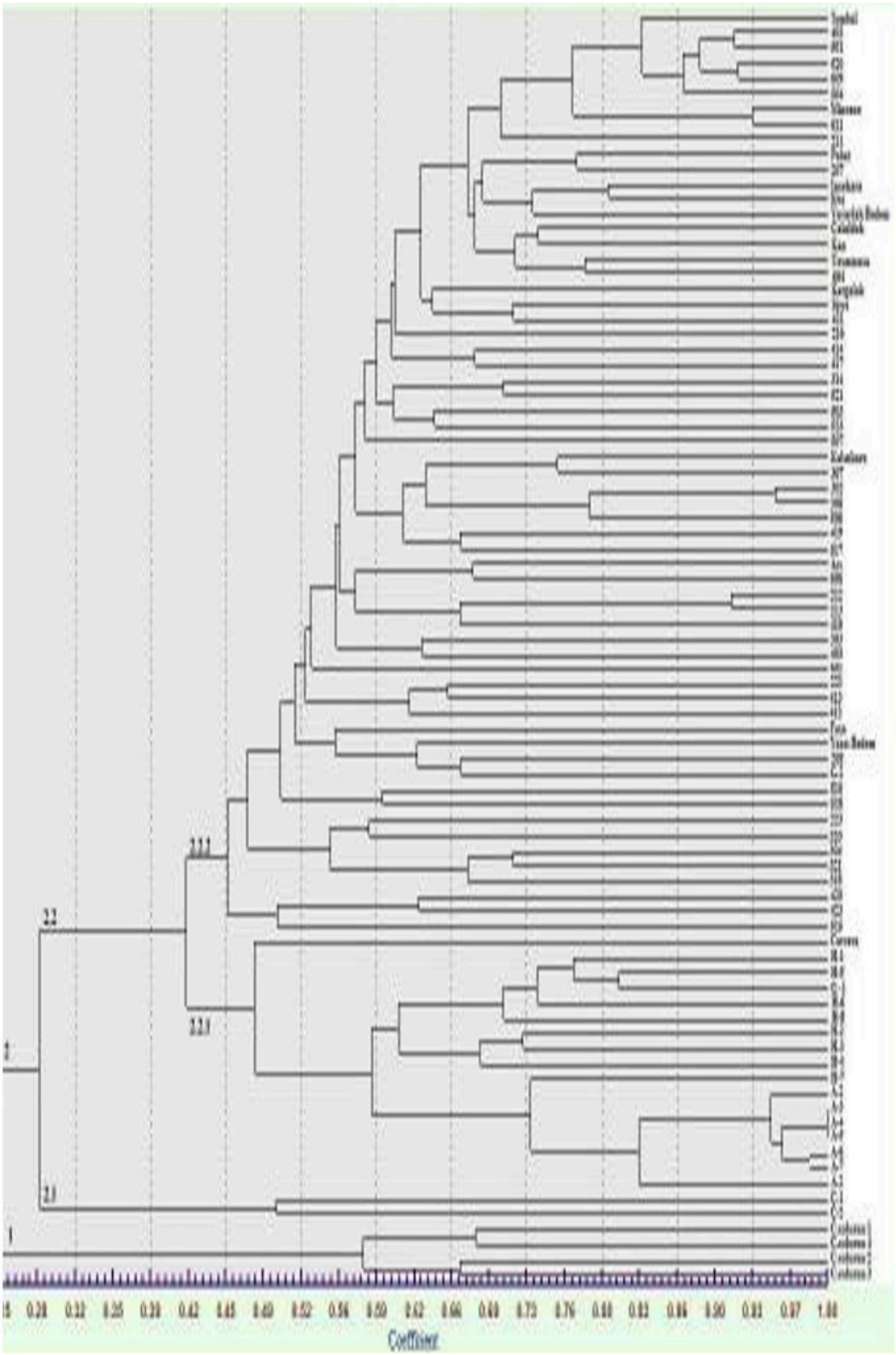

Figure 2. Pedigree obtained according to SSR analysis of hazelnut genotypes

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review
Externally peer-reviewed.

Conflict of interest
The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution
The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval
Ethics committee approval is not required.

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RESEARCH ARTICLE

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Effects of green pruning and combine microelement applications on bud fruitfulness, vegetative development and cluster characteristics of 'Trakya İlkeren' grape cultivar

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Abstract

Green pruning practices are frequently used in viticulture. These practices provide better light penetration, successful air circulation, prevents air humidity and aids disease control in the grapevine canopy. However, excessive leaf removal inhibits vine growth and good fruit ripening. In this study, the effects of some foliar and canopy management practices on cluster, vegetative growth traits and bud fruitfulness of the following year were investigated. Some green pruning (unproductive shoot removal, leaf removal, shoot tip removal, shoot topping), foliar microelements and green pruning + microlements treatments were applied in 'Trakya İlkeren' grape cultivar. Microelement application increased cluster (382.1 g) and berry weight (4.4 g), berry length (19.7 mm) and width (18.2 mm), cluster length (19.5 cm) and width (13.9 cm), berry volume (3.30 cm3) and berry firmness (7.46 N). The highest maturity index was detected in the control group (23.4). While the most intense L* color value was determined in the green pruning + micro element application, the b* color value was determined in the green pruning application. It was determined that leaf area (211.2 cm2), leaf chlorophyll index (32.75 SPAD), shoot diameter (11.13 mm), shoot length (194.71 cm) and internode length (76.81 mm) increased with microelement application. Among the treatments, the most effective application on bud fruitfulness in the following year was at microelement application (1.34 clusters). The effects of the treatments on the amount of Total soluble solids pH, titratable acid and a* color value were not significant. In this study; green pruning + microelement application was recommended for yield and quality sustainability. According to bud fruitfulness results, the highest cluster numbers per node were obtained from pruning with 4 to 10 buds. It is recommended to prune medium or long to obtain higher yields from this cultivar.

Keywords: Bud fruitfulness, Grapevine, Microelements, Quality, Green pruning

INTRODUCTION

The ecological conditions (climate and soil characteristics) of the area where the vineyard is established and different cultural processes, including canopy management, are important on the quality of the grape growing (Van Leeuwen et al., 2019; Bettenfeld et al., 2022). When it comes to quality in table grapes, it refers to the combination of the pleasant taste, the original color of the variety, fully ripe berries and the standard structure bunches (Defilippi et al., 2019; Piernas et al., 2022).

During the development period, multiple factors are effective on the grape

quality. Green pruning, which involves the removal of shoots, leaves, clusters or fruits during the leafy period of the vines, is as important as yield pruning in order to obtain a consistently high-quality crop in vineyards (Winkler et al., 1974; Palliotti et al., 2014; Conti, 2019). In viticulture, a physiological balance is established between the vegetative and generative development of the vine through pruning procedures. Thanks to this balance, it is possible to obtain grapes of sufficient quality and quantity from the vineyards for many years. In order to fully achieve this goal, winter pruning (dormant pruning), which is one of the pruning methods and is done during the vine's full rest period, that is, when the vine's buds burst and its leaves completely shed, in other words, the vegetation of the vine is green pruning is also applied during the period (Salvi et al., 2021).

Green pruning or summer pruning refers to the processes such as leaf removal, pinching and topping, cluster thinning, removing applied to the vegetative parts and bunches of the vines when they are leafy, that is, during the vegetation period. Korkutal et al. (2022) reported that the application of leaf removal and shoot tip removal in the 'Michele Palieri' grape cultivar increased the berry size and caused a decrease in the yield per vine. Korkutal et al. (2018) reported that shoot tip removal during the berry setting period had a positive effect on the berry characteristics of the 'Merlot' grape variety. The main purposes of green pruning are to improve the product quality, to limit the longitudinal growth of the older branches and shoots of the vine, to ensure that the shoots become mature and lignified, to facilitate air flow in the inner parts of the vine, that is, between the shoots and leaves, and to create the necessary sunlight environment around the clusters (Sadeghian et al., 2015). Green pruning applications contain removing unproductive primary and lateral shoots from the canopy, removing shoot tips, thinning clusters, and removing leaves to allow varying levels of sunlight exposure and air ventilation inside the canopy (Senthilkumar et al., 2015; Ye et al., 2022). Removing basal leaves is one of the most common canopy management practices in vineyards (Dry, 2000; Austin and Wilcox, 2011; Di Profio et al., 2011; Silvestroni et al., 2019; Tarricone et al., 2020). This process is often done during the ripening season to improve berry color and aroma, reduce the effects of diseases if there is shading in the canopy due to excessive leaves (Bledsoe et al., 1988; Percival et al., 1994). Low light penetration in the canopy reduces the formation of primorida in the buds. This is likely mediated by carbon availability and assimilation support for the buds (Keller and Koblet, 1995; Dry, 2000; Lebon et al., 2008; Vasconcelos et al., 2009).

Another important cultural practice in viticulture is soil or leaf fertilization. Plants are living organisms that survive depending on the soil. For this reason, the presence of the required level of nutrients in the soil is very important for the development and survival of plants. Grapevine, which is a cultivated plant, takes certain amounts of macro and micro nutrients from the soil every year, ensuring the continuity of growth, development and productivity for many years. The effects of microelements on the yield of different grapevines have been investigated by many researchers (Morshedi, 2001; Domagała-Świątkiewicz and Gąstoł, 2013; Abd El-Razek et al., 2015; Ashoori et al., 2015; Al-Atrushy, 2019). These nutrients are generally found in agricultural soils (Schreiner et al., 2006; Arrobas et al., 2014; Leibar et al., 2017). However, the amount of these nutrients may not always be the amount required for the plant. If the nutrients needed by the plant are deficient in the soil, there is a decrease in productivity and quality. For this reason, in order to maximize efficiency and quality in production and maintain this level, organic and inorganic fertilizers containing one or more macro and micro nutrients must be applied to the soil or directly to the plant. High yield and quality in plants are directly related to the presence of the necessary nutrients in the plants at the required level, and these micro and macro nutrients must be present in the structure of the plant at the required rate in order to obtain the highest desired yield (Marschner, 1995).

There is more than one factor that affects the fruitfulness of grapevine buds according to the node where the winter buds are located in the nodes of the annual shoots. These factors may be related to genetic structure or external factors. Factors such as the grape variety produced, soil and climate characteristics of the growing area, exposure to sunlight, technical and cultural practices applied to the grapevines also affect the formation of flower primordia in winter buds (Srinivasan and Mullins, 1981; Reynolds and Heuvel, 2009; Keller, 2010; Li Mallet et al., 2016). Between 0 and 4 clusters can form in the primary buds of *Vitis vinifera*'s winter buds, but 4 clusters are rare. The number of inflorescences in the primary bud can generally vary between 1 and 2 clusters. In years when all conditions are as desired in vines (climate, growing conditions, nutrition, etc.), it is normal for some primary buds to have 3 cluster buds. Some primary buds may not even produce a single cluster (Dry, 2000; Clingeleffer, 2010; Keller et al., 2004; Vasconcelos et al., 2009). Winter buds on one-year-old canes are closely related to the grape yield of the vine and therefore the total vineyard area (Sánchez and Dokoozlian, 2005; Ulmer et al., 2020). Since the number of clusters to be formed in vines may vary depending on the location of the winter buds on an old stem, determining the productivity of winter buds in different nodes is important in terms of grape yield from the vines. It is necessary to determine the pruning levels that will provide the highest yield and best quality according to the productivity of the winter buds of grape varieties (Rosner and Cook, 1983; Eltom et al., 2014). Knowing which node of a winter cane has the highest bud productivity aids in both planning the number of buds to leave on the vine during yield pruning and determining the level at which the canes should be prune. Productivity status of the buds located in different nodes; it is detected by applying different

methods such as binocular microscope, sectioning of winter buds with a microtome, leaving winter buds in green shoots to grow on vines in the field in summer, counting the number of buds formed by growing cuttings containing single buds in controlled environments outside the field, or counting the buds on long pruned old branches in the field (Antcliff and Webster, 1955; Ferrara and Mazzeo, 2021; Monteiro et al., 2021, 2022; Uray et al., 2023).

In this study; the effects of green pruning and combine microelement applications on shoot development, bud fruitfulness and cluster characteristics were examined in 'Trakya İlkeren' grape cultivar. It is aimed that the results obtained in the study will benefit the development of green pruning applications, the pruning levels of the vines and the continuity of the productivity to be obtained from the vineyards.

MATERIALS AND METHODS

Plant Materials

This research was carried out during 2019-2020 years in the Application and Research Vineyard area of Ondokuz Mayıs University Faculty of Agriculture. The vineyard is located in Samsun province, between 41° 21' 52" N latitude and 36° 11' 29" E longitude, approximately 195 m above sea level and 2.8 km away from the coast. In the study, 'Trakya İlkeren' grape cultivar was used. It's very early ripening, rounded and with purplish-black colored berries, consumes as table and brine leaf, grafted onto 5C rootstock, given a double-cordon training form, 14 years old, planted at a distance of 3 x 1.5 m, short pruned, and fertilized with 20-20-0 compound fertilizer as standard in March-April.

Soil Properties of the Research Vineyard

The soil of the trial area has a heavy clay structure and its pH was determined to be slightly acidic, unsalted and very slightly calcareous. In addition, it was determined that the phosphorus value of the trial area was low, the potassium value was high and the amount of organic matter was moderate. The soil characteristics of the trial area are given in Table 1.

Table 1. Soil characteristics of the research vineyard (0-30 cm)

Feature	Amount	Content Class
Soil texture	110	Heavy clay
pH	6.16	Slightly acid
% Lime ($CaCO_3$)	0.40	Slightly calcerous
% Total Salt	0.052	Unsalted
Phosphorus (P_2O_5 Kg/da)	4.36	Little
Potassium (K_2O Kg/da)	91.0	More
% Organic Matter	2.98	Middle

Phenological Observations of the Vines

In the study, bud burst (EL 4), full bloom (EL 23), veraison (EL35), maturity (EL 38) and dormant (EL 47) dates were determined according to Lorenz et al. (1995). The dates of bud-burst, flowering, veraison, maturity and dormant periods are given in Table 2.

Table 2. Phenological periods of 'Trakya İlkeren' cultivar

Phenological periods	Start	Finish
Bud-burst (EL 4)	15.04.2020	05.05.2020
Blooming (EL 23)	05.06.2020	20.06.2020
Veraison (EL 35)	20.07.2020	07.08.2020
Maturity (EL 38)	17.08.2020	24.08.2020
Dormant (EL 47)	9.11.2020	10.12.2020

Bud-burst in the buds took place between 15.04.2020-05.05.2020. The flowering period started on 05.06.2020 and was completed on 20.06.2020. Veraison stage took place between 20.07.2020-07.08.2020. The maturation period started on 17.08.2020 and ended on 24.08.2020. It was determined that the vineyards entered a full dormant period on 10.12.2020. Visuals of the phenological periods of the 'Trakya İlkeren' grape cultivar are given in Figure 1.

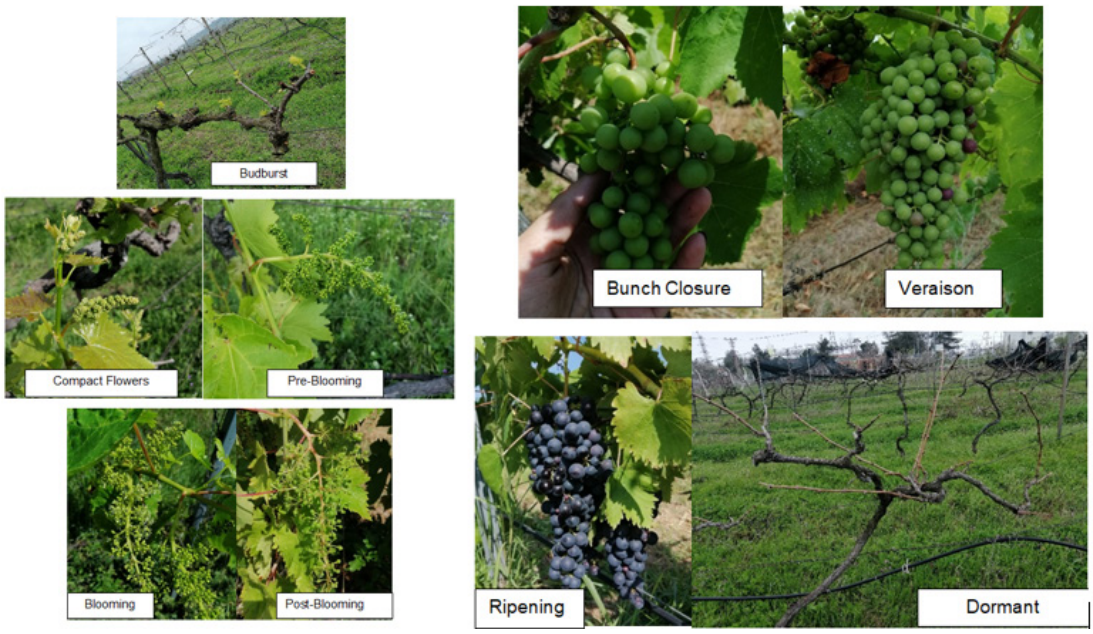

Figure 1. Phenological terms in 'Trakya İlkeren' grape cultivar.

Method

The study was carried out in 4 applications and totally 36 vines were used in all application: Control, combined microelement (ME), green pruning (GP) and green pruning + microelement (GP+ME).

1- Control: In this application, no treatment was applied to the vineyards.

Microelement application: No green pruning was applied to the vines. The combined microelement was applied at compact flowers (EL 15), pre-flowering (EL-22), post-flowering (EL 25) and veraison (EL35) stage. A total of four periods were applied: the compact flowers period (EL 15 stage), before flowering (1 week before blooming), post- flowering (berry set period) and the veraison period. It was applied to the leaves by spraying in liquid form.

2- Microelement application (ME) was applied as at 150 mg/100 L per decare in each period. Composition of combine microelement contents as below;

Water Soluble Copper (Cu-EDTA); 1%,

Water Soluble Iron (Fe-EDTA); 7%,

Water Soluble Manganese (Mn-EDTA); 5%,

Water Soluble Molybdenum (Mo-EDTA); 0.05%,

Water Soluble Zinc (Zn-EDTA); 7%,

Water Soluble Boron (B-EDTA); 1%

3- Green pruning applications (GP): a-removing unproductive shoots that do not have clusters in the early period; b-cutting of the shoot tips before flowering; c-removing leaves at least two leaves at the end of flowering, hard and green berry period and during the veraison period; d-shoot topping was applied when the growth of the shoots stopped and the bottom parts of the shoots started to lignify. a- Unproductive shoot removal: It was applied in the early period by removing all non-cluster shoots. It is the process of removing shoots that reach 15-20 cm in length and do not consist any clusters from the vine. Visuals of the application are given in Figure 2.

b- Leaf removal: The leaves at the bottom of the clusters were manually removed three times, at the end of flowering, during the hard green berry and veraison period. Leaf harvesting was done with at least two leaves in each period. Visuals of the application are given in Figure 3.

c- Shoot tip removal: Tip removal was done before flowering, when the shoots were 40-45 cm. The application was carried out by cutting off 2-3 young leaves from the tip of the shoots. Visuals of the application are given in Figure 4.

d- Shoot topping: It is done in the period when the growth of the shoots stops and the bottom parts of the shoots

start to become lignified. In our study, this period coincided with 25.07.2020. The parts of the shoots above the second laying wire were cut by 30 cm and crowning was performed. The tipping process is defined as shortening the tops to a depth of 30-60 cm when the shoots reach a length of 90-100 cm.

4- Green pruning + microelement application (GP+ME): Microelement and green pruning applications were applied together in grapevines. The solution prepared as 150 g/100 L per decare is sprayed in liquid form on the leaves with a back sprayer during the compact cluster period EL-15 (10.05.2020), before flowering EL-22 (29.05.2020), post flowering EL-25 (26.06.2020) and in the veraison period EL-35 (20.07.2020).

Investigated Features

The effects of the applications were determined by examining the following features. The berry and cluster characteristics of the harvested grapes were determined by randomly selecting three clusters per vine from each application, in a total of 108 clusters were used. Yield (kg vine^{-1}), cluster weight (g), cluster length and width (cm), cluster weight (g), internode length (cm), berry weight (g), berry width (mm), berry size (mm), berry volume (cm^3), berry firmness (N), pH, TSS (^{o}Brix), titratable acidity (TA g/L), maturity index (^{o}Brix/TA) were examined in the grape clusters.

Berry Skin Color Measurement

For color measurement, color changes in the berry skin were determined with a CR-400 Minolta brand color measuring device. Color measurements were made on 20 randomly selected berries in the clusters taken from each vine replicate. CIE LAB (L*, a* and b*) values of the samples were measured with the Konika Minolta CR400 (Minolta, Osaka, Japan) color measuring device.

Shoot and Leaf Features

Leaf area (cm^2), leaf SPAD, shoot length (cm), internodes (mm), cane diameter (mm), periderm development of annual shoots (%) were examined as a result of the applications. In order to determine the effects of the treatments on the leaf area, the width and length of a total of 90 leaves in 9 vines belonging to each application were measured with a ruler during the berry and veraison periods. Leaf area was calculated according to the formula Elsner and Jubb (1988).

$LA= [-1.41 + 0.527(W^2) + 0.254(L^2)]$ (LA: leaf area; W: leaf width; L: leaf length).

Leaf SPAD values were determined by measuring 180 sun-exposed, healthy leaves located in the middle part of the shoot (Konica Minolta SPAD-502) in three replicates from each application during the big green pea and veraison periods.

Evaluation of Bud Fruitfulness

Following the treatment year, the effects of the treatments on the bud fruitfulness of the vines compared to the control group were determined in the vineyard after the dormant buds sprouted. For this purpose; during pruning in March, 5 shoots containing 10 buds were left on 3 vines for each treatment. The number of clusters in each node was counted from the base upwards on the newly emerged shoots until May. The clusters were averaged and the average number of clusters per node was determined from basal to upper node.

Statistical analysis

This experiment was conducted in a randomized block design with four treatments, three replication and three vines for each replication. A total of 36 vines were used in all application. To be determine bud fruitfulness; during pruning in March, five shoots containing ten buds were left on three vines for each treatment. The data obtained were subjected to ANOVA test using SPSS 21.0 statistical program and the differences between the averages were compared at the 5% level according to the Duncan's Multiple range test.

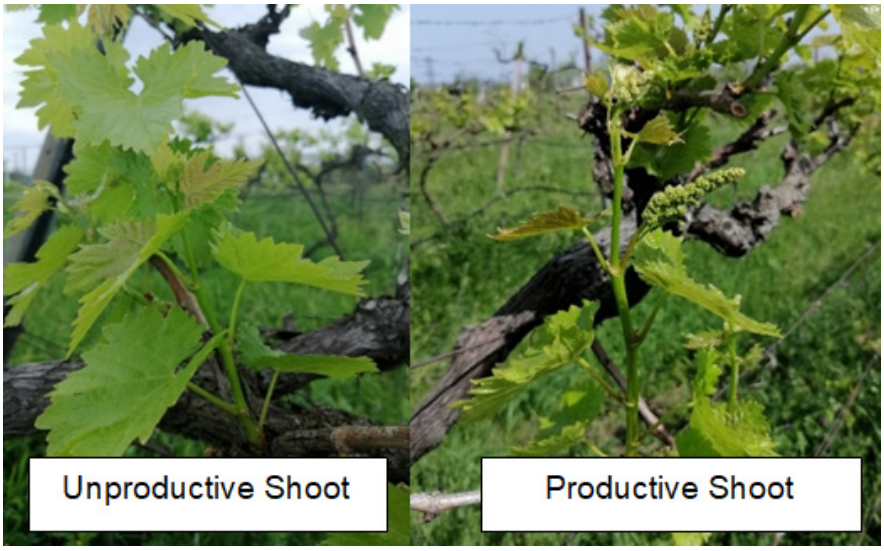

Figure 2. Productive and unproductive shoots

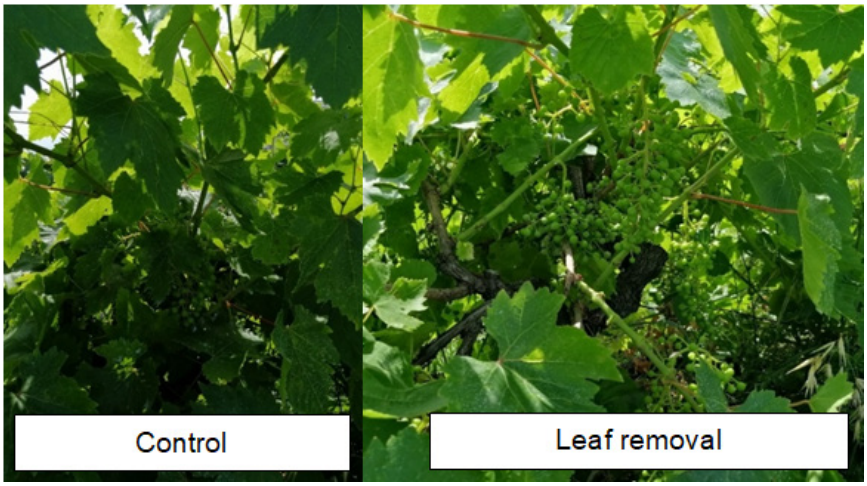

Figure 3. No leaves removed and leaves removed

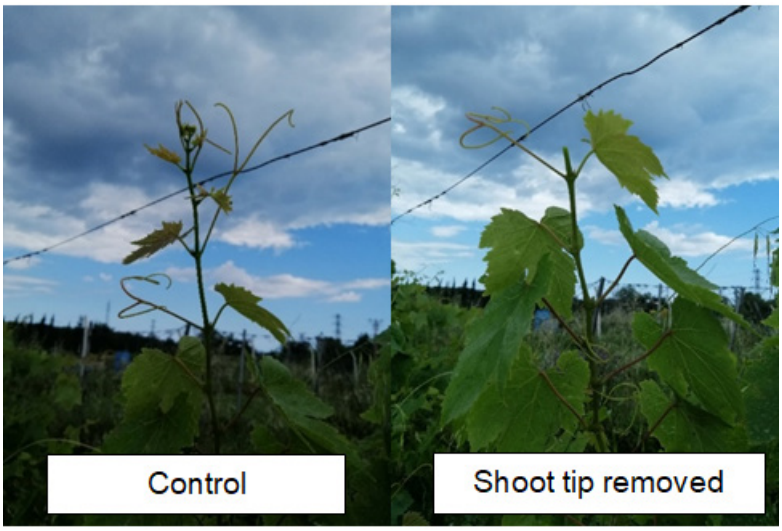

Figure 4. Control and shoot tippping

RESULTS AND DISCUSSION

Green pruning has been making in the summer months for its benefits such as ventilation, light penetration and disease control. On the other hand, it has the opposite effect such as reducing the photosynthetic capacity of the vine, reducing berry color development and delaying berry ripening. The effect of the treatments on cluster and berry characteristics was significant (P≤0.05). The effects of the applications showed significant differences compared to the control group. Among the applications, especially microelement application provided a significant increase in whole clusters and berries (Table 3). In the research, microelement application increased the all cluster and berry features. The highest cluster (382.12 g) and berry weight (4.37 g), vine yield (4516 g), cluster length (19.45 cm) and width (13.90 cm), berry length (19.65 mm) and width (18.17 mm) were obtained from microelement application. On the other hand, microelement when combined with green pruning applications, increased the all cluster and berry features, albeit partially, while the application of green pruning alone decreased the berry weight compared to control group. While the highest cluster weight was obtained in the microelement application with 382.12 g, the lowest cluster weight was obtained in the control group (229.40 g). In the research, it was determined that microelement application increased bunch weight. It was also determined that microelement application had a positive effect on cluster weight when combined with green pruning. The value, which was 266.67 g in the green pruning application, was determined to be 327.91 g in the green pruning + micro element application (Table 3).

Table 3. Effect of green pruning and microlement applications on cluster, berry and yield characteristics

Treatments	Cluster weight (g)	Berry weight (g)	Yield (g^{-1} vine)	Cluster length (cm)	Cluster width (cm)	Berry length (mm)	Berry width (mm)
Control	229.40 d*	4.00 ab	2935.9 b	17.32 c	12.81 b	18.40 b	16.91 c
Green pruning (GP)	266.67 c	3.92 b	3554.5 ab	18.10 bc	12.73 b	17.93 c	16.85 c
Microelement (ME)	382.12 a	4.37 a	4515.5 a	19.45 a	13.90 a	19.65 a	18.17 a
GP+ME	327.91 b	4.10 ab	3825.8 ab	18.82 ab	12.92 b	18.71 b	17.52 b
Standart Errors of The Mean's	6.45	6.50	232.61	0.21	0.08	0.07	0.07
P≤0.05	*	*	*	*	*	*	*

*Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

In this study, green pruning application decreased cluster and berry weight compare to the control vines. Köse et al. (2018) reported that removing fewer leaves increased the cluster and berry weight, TSS and maturity index while removing excessive leaves decreased those parameters in the 'Trakya İlkeren' grapevines. Contrary to the results found in the study, Uslu (1981) found that 25% leaf removal in 'Müşküle' grape increased the berry weight compared to the control and 50% leaf removal. Leaf removal has been reported to increase pH while significantly reducing titratable acidity, TSS, bunch weight, berry size, potassium uptake, and grapevine photosynthesis (Uslu, 1981; Pereira et al., 2006). It has been stated that the removal of leaves showing low photosynthetic activity increases the sugar level in the berry by increasing the light intensity entering the canopy, while it decreases the titratable acidity, pH and K+ levels in the grape juice (Hunter and Visser, 1990). Removing the leaves around the cluster is one of the methods frequently applied in vineyards. In viticulture, leaf removal is done on vines to increase air circulation and light within the canopy. Thus, thanks to the penetration and effectiveness of fungicide sprays, the risk of fungal disease development, especially bunch rot, is reduced (Poni et al., 2006).

Köse et al. (2018) found that removing fewer leaves increased the berry weight in the 'Trakya İlkeren' cultivar. Dardeniz et al. (2018) reported that shoot topping increased berry weight in 'Yalova Çekirdeksizi' cultivar. Contrary to our results found in the study, Uslu (1981) found that 25% leaf removal in 'Müşküle' grape cultivar increased the berry weight compared to the control and 50% leaf removal. And also, Akçay (2013) found that removing fewer leaves increased the berry weight of the 'Sultani Seedless', while removing more leaves decreased it.

Köse et al. (2018) found that removing fewer leaves increased cluster weight, while removing more leaves decreased cluster weight in 'Trakya İlkeren' grape cultivar. Morris et al. (2004) reported that cluster shoot and cluster thinning in 'Aurore', 'Chancellor' and 'Villard Noir' grapes had little effect on cluster weight in all three cultivars. Teker and Altındisli (2021) stated that leaf removal does not significantly affect the yield in the 'Sultani Seedless' grape; however, they reported that 50% leaf removal increased yield compared to 25% leaf removal. Iacono and Sparacio (1999) found that shoot tipping did not affect the yield of 'Cabernet Sauvignon' cultivar.

In the study, the effect of the treatments on berry properties, berry firmness, berry volume and maturity index were found to be statistically significant (P≤0.05). Berry firmness was positively affected by all treatments except the control

group. Although all treatments except control vines were in the same group, microelement application gave the highest berry firmness (7.76 N). The berry volume was determined to be the highest (3.30 cm^3) in the grapevines to which microelements were applied. In the conducted studies, Akgül et al. (2007) found that as a result of the application of different zinc (Zn) fertilizers to the foliar at different doses (0.25%, 0.50% and 0%) in the 'Sultani seedless' grape cultivar, the hardest grape berries were obtained at the 0.25% dose level (785 g), while the soft berries were in the control group. Akural (2016), in 'Alphonse Lavallee' cultivar, the highest berry flesh firmness was determined in the leaves taken at berry setting, from the application of top picking from 40 cm of shoots.

TSS content was not found to be significant among the applications, however, the highest TSS was detected in the GP+ME application (17.8%). While there was no significant difference between titratable acidity treatments, the lowest TA was detected in the control group of grapevines (0.78 $g.L^{-1}$). On the other hand, the maturity index showed significant differences between treatments, the highest was determined as control group vines (23.41), and the lowest was determined as GP (20.39) and ME (20.77) applications (Table 4). The effect of green pruning practices on yield and quality varies depending on the structure of the soil, the climate of the growing region and the type of vine grown (Lanyon et al., 2004; Pellegrino et al., 2014; Reynolds, 2022). Arnold and Bledsoe (1990) investigated the effects of leaf removal at different times and at different intensities on the aroma and flavor of the 'Sauvignon Blanc' grape variety. Vasconcelos and Castagnoli (2000), in a study they conducted on the 'Pinot noir' grape variety; They applied different canopy management methods such as shoot topping at the full blooming stage, cutting the axil shoots and removing the leaves in the cluster area. It was determined that topping application increased the fruit set rate, cluster weight, yield per shoot, leaf size and the contribution of main leaves to the total leaf area; it reduces the total yield per vine, pH, leaf area and pruning wood weight. On the other hand; It was determined that leaf removal application four weeks after flowering had no effect on yield components. If the nutrients consumed by the vine every year through pruning and harvesting cannot be replenished, decreases in the yield and quality of grapes are observed from year to year (Schreiner et al., 2006; Schreiner, 2021; Verdenal et al., 2021). An indispensable condition for successful cultivation is to constantly supply the plant with nutrients through fertilization (Bergman, 1992).

Table 4. Effects of green pruning and microlement applications on berry characteristics

Treatments	Berry firmness (N)	Berry volume (cm^3)	TSS (Brix %)	Titratable acidity ($g.L^{-1}$)	pH	Maturity index
Control	6.71 b*	2.65 b	17.3	0.78	2.96	**23.41 a**
Green pruning (GP)	7.21 a	2.72 b	17.6	0.84	2.98	20.39 b
Microelement (ME)	7.46 a	3.30 a	16.8	0.80	2.90	20.77 b
GP+ME	7.35 a	2.81 b	17.8	0.82	3.03	21.48 ab
Standart Errors of the Mean's	0.05	3.57	0.26	0.01	0.03	0.43
P≤0.05	*	*	ns	ns	ns	*

*Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

The berry skin color value has shown significant differences according to applications (Table 5). According to applications, the least L* value was obtained from the ME application (24.76) in the study. The highest L* value was at GP and GP+ME applications (31.34 and 31.36, respectively). Because of the lowest L* value was at ME application; dark skin color berries were given in ME applied vines. The a* value has not show statistical importance between applications. On the other hand, GP application was the highest a* value (8.83) and the lowest was in the control group (4.60). b* value obtaines as negative, and the least b* value was at ME application (-8.41). In the study, chorome and hue color degrees were not showing importance. On the other hand; while chrome value was the highest at GP (11.42), hue value was the highest at ME application (57.82). Compared to the control group, the applications showed that the black color in the berry skin was lightened the most, that is, the brightest berries were obtained from green pruning + micro element application with a value of 31.45, while the micro element application caused an increase in the black color in the berry skin with a value of 24.82. When the data obtained was examined, it was seen that the black color on the berry skin was lightened the most, that is, the brightest berry were obtained from the green pruning + micro element application at 31.45. The value obtained as 28.61 in the control group was determined as 24.82 in the microelement application. As a matter of fact, although it was determined in the study that green pruning increased the L* color value, Köse et al. (2018) reported that removing fewer and more leaves in the 'Trakya İlkeren' grape cultivar decreased the L* color value.

Compared to the control group, the applications showed that the black color in the berry skin was lightened the most, that is, the brightest berries were obtained from green pruning + micro element application with a value of 31.36; while the micro element application caused an increase in the black color in the berry skin with a value of 24.76. As a matter of fact, although it was determined in the study that green pruning increased the L* color value,

Köse et al. (2018) reported that removing fewer and more leaves in the Trakya İlkeren grape variety decreased the L* color value. According to results, the applications were affected berry skin color, especially ME application improve 'Trakya İlkeren' skin color. GP application has adversely effect on grape skin color. It is considered that this situation causes the photosynthesis efficiency to decrease as a result of the decrease in the leaf area and chlorophylle content of the grapevine caused by the GP application and therefore the skin color development to be retarded as a result of adversely affecting the synthesis of anthocyanin (Table 5).

Skin color was measured with the help of Konica Minolta CR-400 chromameter (color measuring device). In color measurement, L* (indicates brightness value, L*=0 indicates black, L*=100 indicates white), a* (+a* value indicates red color degree, -a* value indicates green color degree), b* (+b* value indicates green color degree, -b* value indicates blue color degree) and hue° (indicates what color is according to its degree) color values were determined (McGuire, 1992). Sunlight has been a key factor in enhancing fruit color development in colored grape cultivars (Dokoozlian and Kliewer, 1996; Chorti et al., 2010; Shinomiya et al., 2015). Shading of clusters has been shown to reduce total anthocyanin content in grape berry skin (Chorti et al., 2010; Gao and Cahoon, 2015; Guan et al., 2017). Gao and Cahoon (2005) determined that the anthocyanin amount in the berry skin was negatively affected by 95 % cluster shading. On the other hand, many researchers emphasized that microlements improve grape berry color (Delgado et al., 2006; Ananga et al., 2013; Strydom, 2014; Abdel-Salam, 2016; Abou-Zaid and Shaaban, 2019; Chen et al., 2020; Abou El-Nasr et al., 2021).

Table 5. Effect of green pruning and microlement applications on berry skin color values

Treatments	L*	a*	b*	Chrome	Hue angle
Control	28.56 b*	4.60	-6.77 b	8.35	-55.62
Green pruning (GP)	31.34 a	8.83	-5.56 a	11.42	-50.35
Microelement (ME)	24.76 c	5.21	-8.41 c	9.99	-57.82
GP+ME	31.36 a	4.82	-5.86 a	7.66	-50.76
Standart Errors of The Mean's	0.158	1.07	0.10	1.065	0.599
P≤0.05	*	ns	*	ns	ns

*Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

This research has shown that the applications significantly affected the average leaf area and leaf chlorophyll content (P≤0.05). In this research, the lowest average leaf area was obtained from vines with control (159.56 cm^2) and GP (156.76 cm^2) applications, while the highest average leaf area was obtained from ME (211.16 cm^2) applied vines (Figure 5). Similarly, in a study conducted by Poni et al. (2006) on 'Sangiovese' and 'Trebbiano' grape cultivars, the effects of early leaf defoliation were examined. It was determined that fruit set, cluster weight, number of berries per cluster, berry size and cluster compactness decreased with all leaf removal applications.

Leaf is one of the important main organs of the vine. Its primary function is to perform leaf photosynthesis and respiration. Leaf removal is one of the most common summer practices in vineyards aimed at an improvement of cluster microclimates, ripening and reducing fungal disease risks. Leaf removal improves cluster exposure, ventilation and the efficiency of pesticide applications (Guidoni et al., 2008). Leaf removal also provides better light penetration, successful air circulation prevents air humidity and helps with disease control in the grape canopy (Sternad et al., 2015). However, excessive removing of the leaves prevents the growth of the vines and the ripening of the fruits well. For this reason, it should be avoided excessive leaf removing during the growth season (Köse et al., 2018).

Similarly, GP application caused a significantly decrease in leaf chlorophyll content (29.56) than even control vines. The highest leaf chlorophyll content was obtained in ME applied grapevines (32.75). The results obtained from the research showed that the average leaf area and leaf chlorophyll content, which decreased with GP application, increased with the addition of ME to GP application (Figure 6). Al-Atrushy (2019) stated that the application of micronutrients significantly increased leaf area, total chlorophyll content, number of clusters per vine, cluster weight and yield per vine, as well as weight and size of 100 berries, and TSS in 'Mirane' grapevines. On the other hand; the needs of the plant can be met with fertilization during the growing period, or at different stages of the plant's development. In order to fertilize both economically and as needed, plant analyses are important to control the fertilization plan and determine the availability of fertilizer applied to the soil, along with soil analysis (Conradie, 2001; Arrobas et al., 2014).

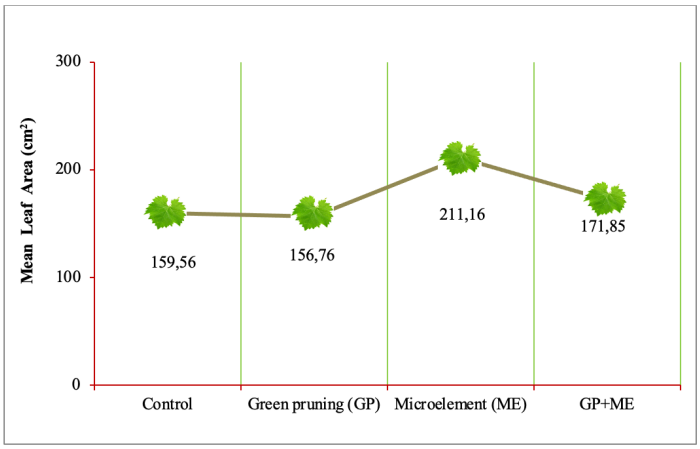

Figure 5. Effect of green pruning and microlement applications on leaf area (cm^2). Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

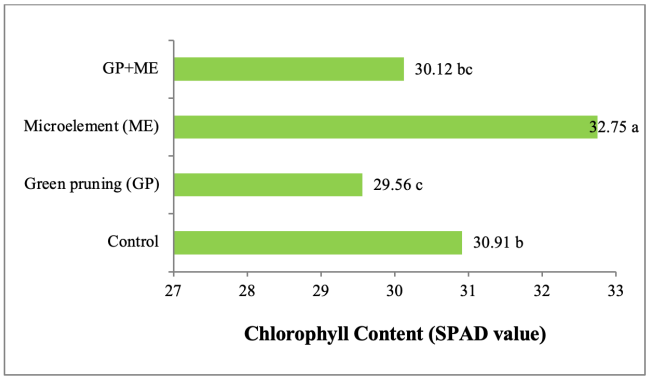

Figure 6. Effect of green pruning and microlement applications on leaf chlorophyll content (SPAD value). Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

Shoot length, cane diameter, internodes and periderm encompassed internodes showed statistically significant differences among foliar treatments (Figures 7, 8, 9 and 10). Among the treatments, microelement application increased shoot length (194.71 cm), cane diameter (11.13 mm) and internode length (76.81 mm). As expected, the lowest shoot growth was observed in GP treated vines. It is seen that microelement application increased shoot growth. Contrary to expectations, ME application delayed periderm development. The highest number of periderm encompassed internodes was found in control vines (7.76). The lowest number of encompassed internodes was obtained from microelement treated vines. A similar result was found by Xu et al. (2020) that the addition of microelements in fertilizer to loquat rootstock seedlings can significantly delay the lignification process of the cambium of grafted vines, which exhibited the greatest improvement in stem thickening.

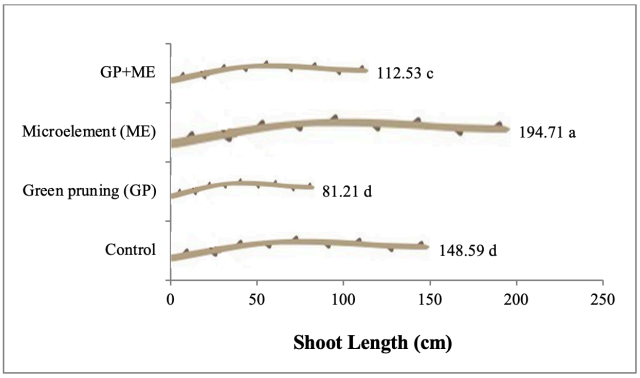

Figure 7. Effects of green pruning and microlement applications on shoot growth. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

Regular (Caspari et al., 1998) or 90% leaf removal (Chaumont, 1995; Ollat, 1998) reduces photosynthesis. Leaves are also important as a food organ for humans, as the products of photosynthesis produced cannot be fully transported from the leaf to other organs during the day (Downton et al., 1987, Düring, 1988; Roper and Williams, 1989). This study showed that green pruning practices reduced the photosynthetic activities of the plant as it caused a decrease in the

average leaf area of the grapevine, a decrease in the chlorophyll index, and a decline in shoot length. As a result, the lack of photosynthetic activity affected the growth and development of grapevines, cluster quality and skin color.

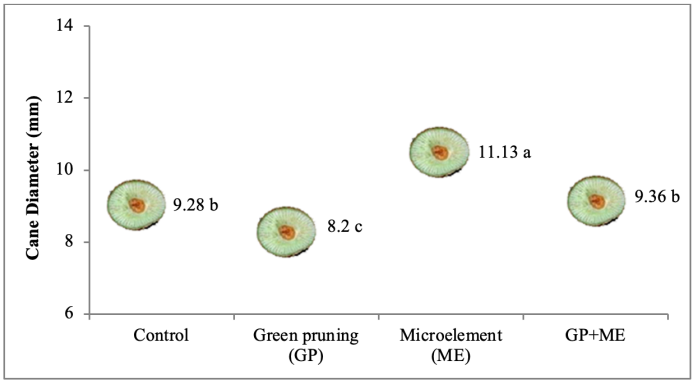

Figure 8. Effects of green pruning and microlement applications on cane diameter. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

It is known that if grapevines cannot meet the plant nutrients they need during their natural development period, the yield obtained from vineyards will decrease and the quality of the products will be negatively affected. Although the amounts of plant nutrients needed by grapevines vary, in general, grapevines need mainly N, P, K, Mg, Ca, S, B, Mn, Cu, Zn and Mo during the development period (Verdenal et al., 2021; James et al., 2023).

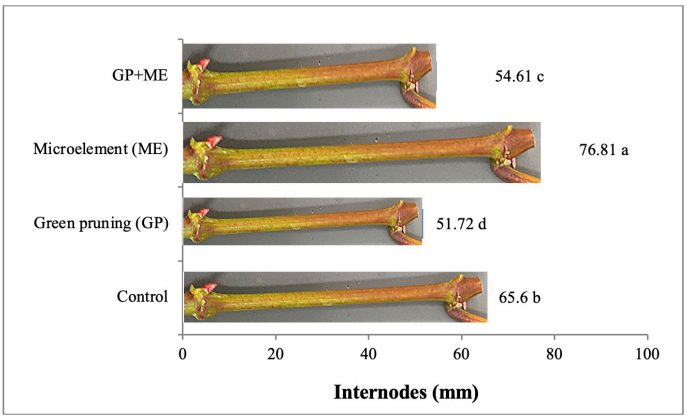

Figure 9. Effects of green pruning and microlement applications on shoot internodes. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

Applying foliar fertilization is a remarkable method in order to maintain or even increase the growth and yield in micronutrient deficiencies encountered in grapevines (Abd El-Razek et al., 2011; El-Boray et al., 2019; Hosseinabad and Khadivi, 2019), especially during the crop season (Masi and Boselli, 2011; Baldi et al., 2017; Gautier et al., 2018; Kumar and Mohapatra, 2021). In this study, microelement application caused a decrease in lignification which has slowed down periderm development.

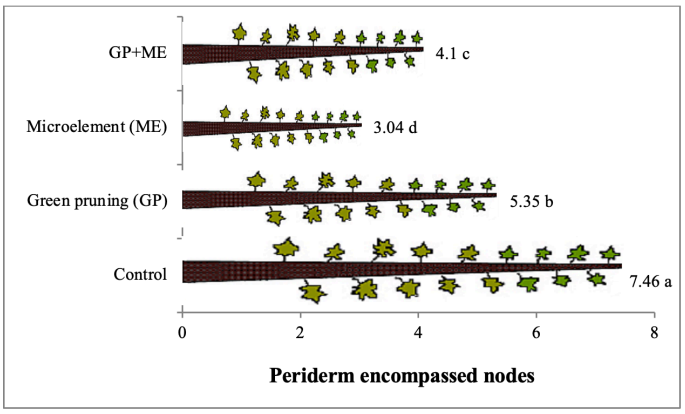

Figure 10. Effect of treatments on shoot development and periderm encompassed nodes. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

In the study, bud fruitfulness was determined to obtain the effcts of the previous year's applications. It was seen that the applications had a significant effect on the bud fruitfulness in the following year (Figure 11). In the study, the effects of the treatments on the bud fruitfulness in the following year were determined by counting the clusters carried by the shoots formed at the nodes from the bottom in May under vineyard conditions. While the average number of clusters calculated based on the average of 10 nodes in the control group vines was determined to be 1.34 clusters, this ratio was calculated to be 1.15 clusters in the GP application, 1.86 clusters in the ME application and 1.45 clusters in the GP+ME application. As can be seen, while green pruning applications (GP) significantly reduced the bud fruitfulness the following year compared to the control vines, ME application significantly increased the bud fruitfulness. On the other hand, the negative effect of GP application caused a significant increase in bud fruitfulness by adding ME to GP application. In the research, according to bud fruitfulness results, it was evaluated that from 4 to 10 buds can be left during pruning in the 'Trakya İlkeren' grape cultivar depending on the preferred training system, which would be beneficial in terms of total vine yield (Figure 11). As a matter of fact, Çelik (2017) reported that in 'Trakya İlkeren' grape cultivar, 4-8 buds can be left in pruning. In our study, it was determined that the number of clusters per bud increased from the 4th nodes.

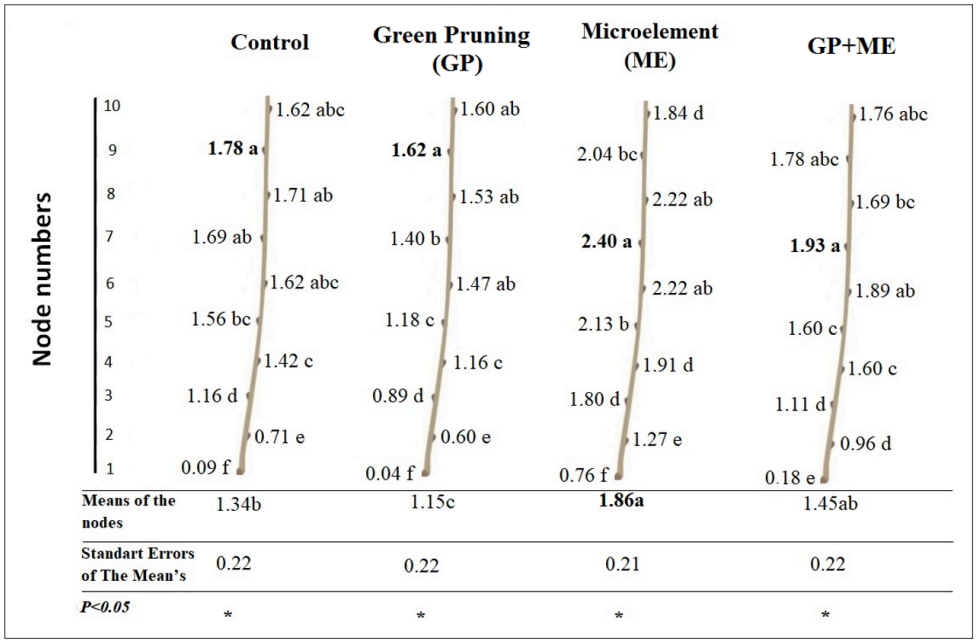

Figure 11. Effect of treatments on the number of clusters formed on the following year's nodes. Means followed by similar letters are not statistically different (P≤0.05) as compared by Duncan's multiple range test.

Several factors may influence bud fruitfulness, such as light, temperature, nutrient and the water status of vineyard (Shikhamany, 1999; Dry, 2000; Mullins et al., 2000). Our study showed that the combined ME application highly affected on bud fruitfulness on grapevines. Especially, when making green pruning application in the vineyards, it should be supported with microelements to prevent decreasing photosynthetic matters. When vineyards show nitrogen, potassium, boron and iron deficiency, it shows a high incidence of bud necrosis and/or low bud fertility (Botelho et al., 2005). Besides, mineral fertilizers have an effect on the growth of shoots, the formation of primordia in winter buds, and leaf covering of clusters. Foliar applications with mineral fertilizers promote the activation of a number of metabolic processes in plants (Aleynikova et al., 2021). For this subject; Mostafa et al. (2017) emphasized that foliar nutrient application (Fulvic acid +Mg + K) gave the highest significant increase in bud burst and fertility, shoot length, leaf surface area, total chlorophyll content, yield/vine, TSS%, total sugars and total anthocyanin content in berry skin while it gave the lowest decrease in acidity compared with that of control vines. Thanks to the green pruning performed during the vegetation period, the incidence of fungal diseases such as Downy mildew, Powdery mildew and *Botrytis cinerea*, which develop rapidly in environments with high relative humidity and in shaded areas, decreases and the contact of the applied fungucides to the inner parts of the vine and the clusters becomes easier (Sholberg et al., 2008, Mahrous and Shalaby 2009; Austin et al., 2011; Almanza-Merchán et al., 2014; de Bem et al., 2015).

CONCLUSION

Although green pruning is one of the most common practices in vineyards, it may have negative effects on the photosynthetic capacity of the grapevine, developmental disorder, yield and quality decline and the productivity of the following year's buds. In this study, the effects of green pruning practices and combined microelement applications on bud fruitfulness, vegetative growth, yield and cluster characteristics of 'Trakya İlkeren' grape cultivar were investigated. It was determined that green pruning practices had negative effects on bud fruitfulness, vegetative growth, yield and bunch characteristics. On the other hand, microelement applications had positive effects on bud fruitfulness. It was determined that supporting green pruning with microelement application will have a mitigating effect on these negative effects. In terms of reducing the negative effects of green pruning practices, it was observed that micro element application contributed positively to the improvement of bud fruitfulness, growth and cluster characteristics. For this reason, it has been observed that microelement application will be beneficial in the period starting from green pruning until veraison in order to reduce the losses of growth and cluster specifications caused by green pruning practices carried out for many purposes such as sun light penetration in canopy, ventilation, control of fungal diseases, berry skin coloration and aroma development in the summer months. When the bud fruitfulness of the following year's is evaluated, it is thought that the highest cluster number per nodes for the 'Trakya İlkeren' cultivar is obtained from pruning with 4 to 10 buds. For this reason, it was concluded that it would be appropriate to perform medium or long pruning and to prefer Lenz Mozer, Guyot or Pergola training systems.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

This study, which was part of the Fatma Türk's MSc Thesis (YOK Thesis No: 720471/Date: 08.02.2022) is no potential conflict of interest was reported by the author(s).

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

The data can be available upon the request.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Changes in bioactive compounds and antioxidant activity of Gaziantep and Kastamonu garlic during black garlic production

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Abstract

Garlic (*Allium sativum L.*), a member of the Alliaceae family, has been widely used in cuisine and traditional medicine since ancient times. Black garlic is produced by fermentation of fresh garlic under controlled conditions for a certain period at high temperature (60-90°C) and high humidity (70-90%). According to the Turkish Statistical Institute (TURKSTAT) data, Kastamonu and Gaziantep garlic varieties are the most cultivated garlic varieties in our country. Changes in protein, sugar content, antioxidant capacity (DPPH and ABTS methods), total phenolic content, 5-hydroxymethylfurfural (HMF) content, and organosulfur compound profiles were investigated in samples taken from Kastamonu and Gaziantep fresh garlic at 7, 14, 21, and 28 days of black garlic production under 65°C temperature and 70% humidity conditions. With these analyses, the differences between black garlic and fresh garlic and the changes in black garlic during the production process were revealed in detail. It was determined that the amount of total phenolic content and antioxidant capacities increased in the black garlic production processes of both regions compared to fresh garlic. While sucrose was fresh garlic's dominant sugar, fructose was black garlic's dominant sugar. Among the organosulfur compounds, allicin was dominant in fresh garlic and SAC in black garlic. It was determined that SAC was formed after the enzymatic conversion of ɣ-glutamyl-S-alk(en)yl-L-cysteine and ɣ-glutamyl and the temperature and fermentation time used in black garlic production increased the formation of SAC. The protein content ranging between 5.8%-7.3% in fresh garlic was 13.1-14.2% in black garlic. Fresh and black garlic from the Gaziantep region was determined to have higher total phenolic content, antioxidant capacity, and organosulfur compound contents.

Keywords: Black garlic, Antioxidant capacity, HMF, Organosulfur compounds

INTRODUCTION

Garlic (*Allium sativum L.*), a member of the Lilliaceae family, is considered to be the most important vegetable produced in this family after onion (Kim et al., 2004, Hamma, 2013). According to FAO data, garlic constitutes 2.4% of the 1.14 billion tons of vegetables produced in the world in 58.3 million hectares in 2020. Worldwide, 28 million tons of garlic are produced on 1.6 million hectares (FAOSTAT, 2020). Garlic is a vegetable grown in many regions of our country and can adapt well to the environmental conditions of our country. According to TURKSTAT data, more than 136 thousand tons of garlic were produced in our country in 2020. Kastamonu province ranks first with 23 thousand tons of dry garlic production.

Garlic and its products are widely consumed due to their unique taste and rich

nutritional value (Condurso et al., 2019; Tao et al., 2016; Zhang et al., 2016; Huang et al., 2015). This versatile ingredient has been a staple in global cuisine for millennia, valued not only as a food item but also for its medicinal properties (Moutia et al., 2018). Garlic contains organosulfur compounds such as allicin, alliin, and ajoene, as well as antioxidant-rich phenolic compounds such as catechin, epicatechin, resveratrol, coumaric, chlorogenic acid, carbohydrates (sucrose, glucose), minerals, amino acids, and vitamins such as vitamin A, B1, B2, niacin, and C (Raghu et al., 2012).

The health effects of garlic are attributed to its bioactive compounds, particularly organosulfur compounds that cause bitterness and a pungent odour. Studies have shown that garlic consumption has potential benefits against chronic diseases such as cancer and diabetes (Oyawoye et al., 2022; Queiroz et al., 2009). The characteristic odor of garlic makes it not widely preferred in the food industry despite its potential health benefits. Many people hesitate to consume garlic because of its odor. Black garlic has been developed as an attractive alternative for consumers who want to enjoy the health benefits of garlic. Black garlic has a milder odor and a more pleasant texture. Black garlic is produced by heat treatment of fresh garlic under controlled temperature (60-90 °C) and high humidity (50-95%) without any extra processing or additives (Zhang, et al. 2016). Many reactions occur during the production of black garlic. Depending on these reactions, carbohydrates, organosulfur compounds, amino acids, polyphenols and other antioxidant compounds in fresh garlic undergo significant changes. As a result of this change, studies have determined that the bioactivity of black garlic is higher than fresh garlic (Kim et al., 2011; Qiu et al., 2020). Various changes occur during the fermentation stages in black garlic production. The most important of these changes is the increase in the amount of S-allyl cysteine (SAC) compound as a result of the fermentation process (Haber et al., 1996; Atanasova-Goranova et al., 1997). During the heat treatment of garlic, the Maillard reaction changes the nutrient content, color, texture, and flavor of garlic (Yuan et al., 2016).

This study aims to investigate the biochemical composition, antioxidant capacity, HMF, and sugar content of fresh garlic grown in the Gaziantep and Kastamonu regions of Turkiye during black garlic production. For this purpose, samples were taken on different days of the fermentation process, evaluations were made and changes in protein and sugar content, antioxidant capacity (DPPH and ABTS methods), total phenolic matter content, HMF content, and organosulfur compound profiles were determined.

MATERIALS AND METHODS

Material

Kastamonu fresh garlic samples were obtained from a local market in Taşköprü city of Kastamonu province and Gaziantep fresh garlic samples from the Araban city of Gaziantep province. The specimens were then subjected to a controlled process of black garlic production, which involved exposure to specific temperature and humidity conditions. During the production of black garlic, fresh garlic samples were subjected to temperatures of 65 ^{0}C, humidity levels of 70%, and kept in a humidity chamber (HCP105, Memmert GmbH + CO, KG, Germany) for 28 days.

Chemicals

Methanol, sodium hydroxide, ethanol, acetonitrile, Folin–Ciocalteu reagent, and formic acid were purchased from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteu phenol reagent, sodium carbonate, Gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, ABD). Sucrose, glucose, and fructose were procured from Merck Company (Darmstadt, Germany). Deionized water was utilized in HPLC to prepare the mobile phases. Standard solutions and other sensitive solutions were prepared daily.

Protein determination

Crude protein content was determined by the Kjeldahl method AOAC (1997).

Sugar determination

For sugar analyses, 2 g of sample was taken, and 20 ml of water was added and extracted with a magnetic stirrer for 30 minutes. At the end of this time, the extract obtained was centrifuged (Hettich Universal 320R) at 5500 rpm at 4°C and the upper clear part was removed and filtered through 0.45 µm filters (Lee and Coates, 2000). The filtrate obtained was injected into the Shimadzu Prominence-I HPLC device and the sugar amounts in the samples were determined. Aminex HPX-87H (300 x 7.8 mm) (Bio-Rad, California, USA) column and 5 mM sulphuric acid solution were carrier phases. The sugar concentrations were determined in the samples by an external standard method using RID (Refractive Index Detector). For this purpose, calibration solutions were prepared from sucrose, glucose, and fructose standards at 5 different concentrations, and sugar amounts were calculated according to the calibration curve.

Determination of antioxidant capacity

Two different methods (DPPH and ABTS) were applied to assess the antioxidant capacity of garlic samples. The ability to suppress free radicals was assessed in a UV-Vis (Agilent Cary 60) spectrophotometer at 515 nm using DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995; Kelebek et al., 2009). The ABTS assay was carried out by the method given by Saafi et al. (2009). By combining it with 2.45 mM potassium bisulfate and leaving it in the dark for 12-16 hours, a 7 mM solution of ABTS (2,2′-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid]) was created. The solution was diluted with sodium acetate (pH 4.5) buffer to an absorbance of 0.70 0.01 at 734 nm using a UV-Vis spectrophotometer. The extract was then combined with 2.98 mL of the prepared buffer and incubated in the dark for 20 minutes at room temperature. The absorbance at 734 nm was then measured with a UV-Vis spectrophotometer (Agilent-Cary 60). The Trolox calibration curve was utilized to calculate antioxidant activity, and the results were expressed as mol Trolox per 100 g.

Total Phenolic Compounds (TPC) Analysis

TPC analysis was performed using the Folin-Ciocalteu reagent method, as described by Singleton and Rossi (1965). A spectrophotometer cuvette was filled with 200 L of the extract/standard solution and 1.5 mL of Folin-Ciocalteu reagent (1:10). After five minutes, 1.5 mL of 6% sodium carbonate solution was added to the tubes, which were then stored in the dark for 90 minutes at room temperature. A UV-Vis spectrophotometer was used to detect absorbance at 765 nm. We generated a 500 ppm gallic acid solution for the calibration curve and reported the results in mg/100 g.

Determination of HMF content

Hydroxymethylfurfural (HMF) levels in fresh and black garlic samples were determined using the method published by Kelebek et al. 2016. In brief, 0.4 g of the substance was mixed with 5 ml of distilled water. The mixture was then concentrated to 10 mL using 100 L of Carrez 1 and 100 L of Carrez 2. The resulting blend was ultrasonically treated for 30 minutes in a water bath before centrifuging at 5500 rpm at 4 °C. The filtrate was directly fed into an Agilent 6430 Triple Quadruple mass spectrometer equipped with high-performance liquid chromatography (LC-DAD-ESI-MS/MS) on a Phenomenex Luna C18 column (250 4.6 mm, 5 m) (Torrance, California, USA). A positive ion electrospray ionization (G1948 B) source is used in the spectrometer (Agilent 6430 LC-MS/MS). The detected ions for the HMF complex were m/z 109 and m/z 127. For quantification, the signal response of an ion with a m/z of 109 was observed. Chromatograms at 285 nm detection wavelength were used to collect data (Kelebek et al. 2016).

Analysis of organosulfur compounds

The extraction of the samples was prepared according to the method of Zhu et al. (2016) with modifications. Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent 1260 LC with a Diode Array Detector was used to identify organosulfur compounds. Organosulfur compounds were determined by LC-DAD-ESI-MS/MS using a Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm) (Torrance, California, USA) in positive mode.

Statistical analysis

Statistical analysis was performed by One-Way ANOVA using SPSS 22.0 (version 22, SPSS Inc., Chicago, IL, USA). Duncan's test measured differences in the content levels of the results and means with *p*-values less than 0.05 were indicated to be statistically significant.

RESULTS AND DISCUSSION

Protein content

The protein contents of fresh garlic grown in the Gaziantep and Kastamonu regions and black garlic produced from this garlic on the 7th, 14th, 21st, and 28th days are given in Table 1. While the amount of protein in Gaziantep and Kastamonu fresh garlic was 5.76% and 7.33%, respectively, it was determined that there was a 2-fold increase in the amount of protein until the 21st day in the black garlic production process, and a decrease was observed after the 21st day. Nassur et al. (2017) reported the protein content of fresh garlic as 10.62% and the protein content of black garlic as 11.75% in their study. Liu et al. (2018) found that the protein content of fresh garlic was 5.32% and the protein content of black garlic was 10.26%.

Table 1. General composition of garlic

	Protein (%)	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	Total sugar (g/kg)	HMF (g/ kg)
Gaziantep fresh garlic	5.86±0.67^{f}	3.43±0.19^{f}	2.81±0.27^{i}	1.35±0.01^{i}	7.60±0.07^{j}	Nd
G7	8.34±0.11^{de}	35.37±0.23^{a}	7.72±0.04^{g}	32.38±0.04^{g}	75.48±0.22^{g}	0.09±0.01^{f}
G14	8.27±0.21^{d}	34.92±0.02^{a}	9.74±0.03^{f}	68.98±0.07^{f}	113.66±0.07^{f}	0.14±0.01^{e}
G21	12.59±0.66^{a}	22.85±0.10^{c}	16.78±0.02^{d}	168.45±0.12^{d}	208.09±1.25^{d}	0.63±0.03^{c}
G28	9.85±0.54^{bc}	17.49±0.01^{d}	21.92±0.01^{b}	219.34±0.10^{b}	258.76±0.01^{b}	0.86±0.01^{b}
Kastamonu fresh garlic	7.33±0.32^{e}	6.18±0.01^{e}	3.29±0.03^{i}	2.29±0.56^{i}	11.76±0.58^{i}	Nd
K7	9.28±0.48^{c}	24.56±0.08^{b}	4.16±0.06^{h}	19.65±0.07^{h}	48.38±0.09^{h}	0.10±0.01^{f}
K14	10.03±0.30^{bc}	35.03±0.02^{a}	12.79±0.05^{e}	95.31±0.02^{e}	143.14±0.05^{e}	0.19±0.01^{d}
K21	13.10±0.06^{a}	24.07±0.01^{b}	19.43±0.01^{c}	189.50±0.01^{c}	233.02±0.01^{c}	0.65±0.02^{c}
K28	9.87±0.13^{bc}	3.58±0.01^{f}	27.44±0.10^{a}	272.86±0.30^{a}	303.89±0.20^{a}	0.95±0.01^{a}

$^{a-h}$Different exponential letters in the same column indicate a significant difference between the samples ($p<0.05$). Nd: Not detected. G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 14 days, G21; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 28 days, K7; Black garlic produced from Kastamonu fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K21; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 28 days.

Sugar content

Carbohydrates constitute 26-30% of fresh garlic and contain 23% fructans. Fructans are a group of oligopolysaccharides consisting of fructose units linked by β-2,1 bond and constitute 70-80% of the dry matter of garlic (Huang et al., 2011; Li et al., 2017). Fructans are hydrolyzed to fructose and glucose by heat treatment applied during black garlic production. Thus, black garlic becomes sweeter and the texture softens (Hofmann et al., 2000; Yuan et al., 2016). The sucrose, glucose, and fructose compounds in fresh garlic from the Gaziantep and Kastamonu regions and black garlic produced from these regions were determined and presented in Table 1. Analysis revealed that sucrose was the predominant sugar in fresh garlic, followed by glucose and fructose. Additionally, it was observed that the levels of sucrose, glucose, and fructose increased with the fermentation period in the black garlic production process, with fructose becoming the dominant sugar after 14 days. Casas et al. (2017) determined the highest increase in fructose (from 0.38 to 44.73 g/100 g/100 g DW) and glucose (from 0.21 to 2.51 g/100 g DW) in black garlic using high-performance liquid chromatography (HPLC). Choi et al. (2008) examined the physicochemical properties of black garlic and determined the fructose, arabinose, glucose, and sucrose compounds of fresh garlic as 63.9 mg/100 g, 51.1 mg/100 g, 91.6 mg/100 g, and 76.3 mg/100 g, respectively. In black garlic, fructose (63.9 mg/100 g), arabinose (114.5 mg/100 g), glucose (181.7 mg/100 g), and sucrose compounds were identified.

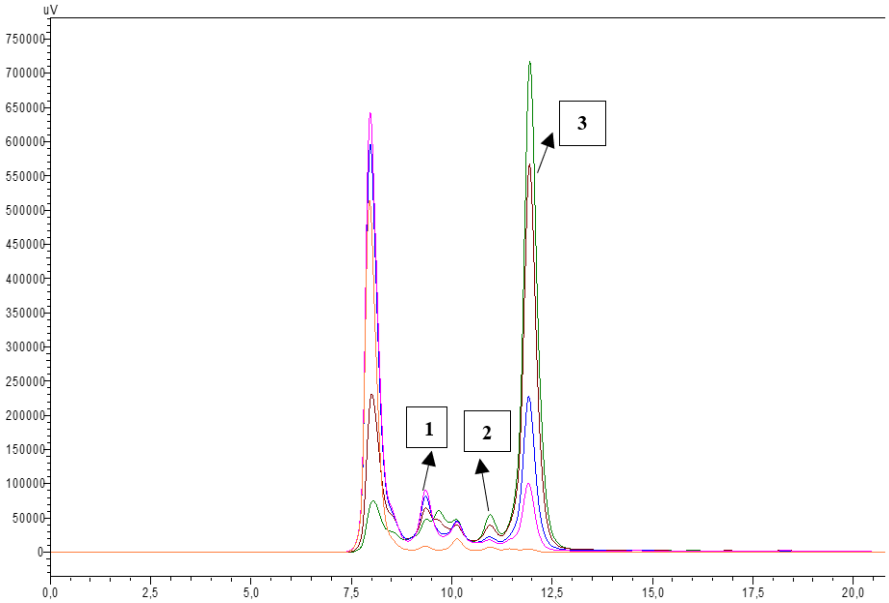

Figure 1. Chromatogram of the sugar determined in garlic (1; Sucrose, 2; Glucose, 3; Fructose)

HMF amount

The amount of HMF was determined during the production process of black garlic, and the data obtained are presented in Table 1. LC-MS/MS was employed for identification, with the study conducted in positive ion mode based on 127>109 multiple reaction monitoring (MRM) transitions. Notably, HMF was not detected in fresh garlic.

During the production process of black garlic derived from garlic cultivated in the Gaziantep and Kastamonu regions, the HMF content was found to range between 0.09 and 0.86 g/kg in Gaziantep black garlic and 0.10 and 0.95 g/kg in Kastamonu garlic. Notably, in some studies, the maximum HMF content in black garlic at the end of the production process has been reported to be approximately 5 g/kg (Zhang et al., 2016).

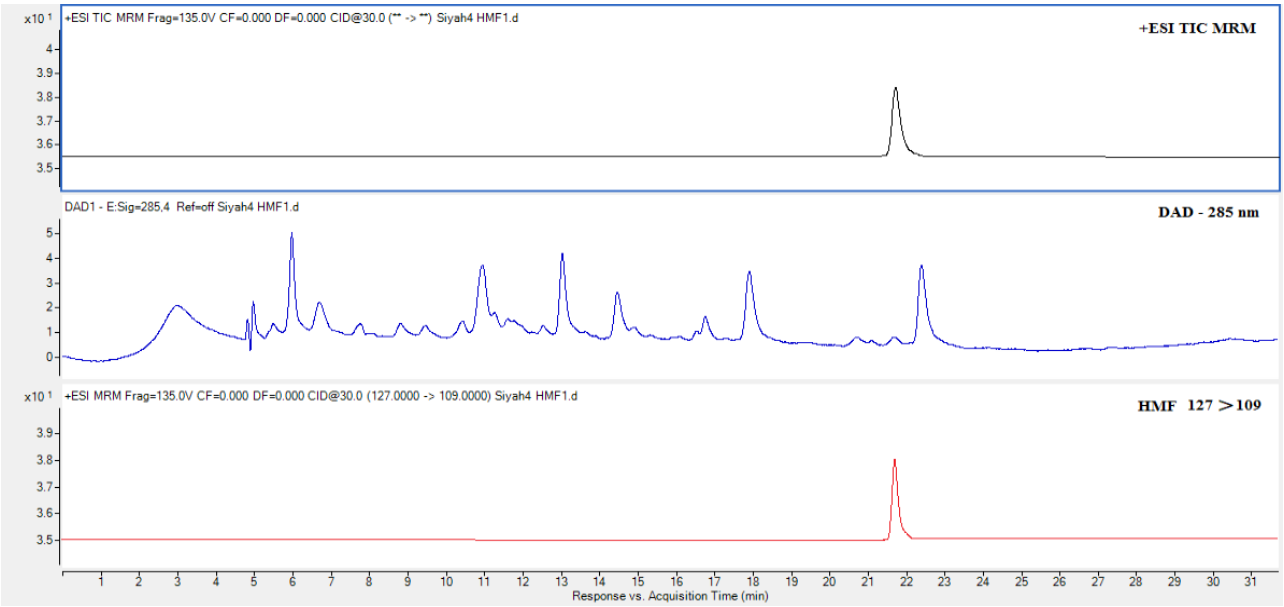

Figure 2. Chromatogram and spectrum obtained for HMF analyses in positive mode

Antioxidant capacity and total phenolic content

The antioxidant capacity of fresh garlic grown in Gaziantep and Kastamonu regions and black garlic produced from this garlic were determined by two different methods, DPPH and ABTS, on the 7th, 14th, 21st, and 28th days (Table 2) and a statistically significant difference was observed ($p<0.05$). The antioxidant capacity potential of fresh garlic grown in Gaziantep and Kastamonu regions was determined as 53.69 µmol Trolox /100 g and 56.85 µmol Trolox/100 g by the DPPH method and 188.14 µmol Trolox /100 g and 109.55 µmol Trolox /100 g by ABTS method, respectively. It was determined that antioxidant capacity increased in the black garlic production process depending on the increase in production time. It was determined that the antioxidant capacity of black garlic produced from fresh garlic grown in the Gaziantep region was higher than that of black garlic produced from fresh garlic grown in the Kastamonu region.

The prominent feature of black garlic is its potent antioxidant activity. Studies show that black garlic can effectively scavenge DPPH, ABTS, hydroxyl, nitrite, and superoxide anion radicals (Jeong et al., 2016; Kim, Nam, et al., 2012; Lee et al., 2009; Zhang et al., 2016). Black garlic plays a similar regulatory role in lipid peroxidation and superoxide dismutase activity, with its effect on reducing free radical production (Bae et al., 2014; Kim, Nam, et al., 2012; Sato, Kohno, Hamano et al., 2006). In particular, antioxidant activity increases during the processing of black garlic. This increase is due to the formation of new antioxidant compounds such as S-allyl cysteine (SAC), polyphenols, carboline derivatives, melanoidins, Amadori and Heyns compounds along with increases in the amounts of compounds such as HMF and pyruvate (Qiu et al., 2020). This reinforces the unique antioxidant properties of black garlic.

Phenolic compounds are aromatic or aliphatic compounds characterized by at least one aromatic ring to which one or more hydroxyl (OH) groups are attached. The anti-inflammatory, anti-aging, antiproliferative, and antioxidant properties of phenolic compounds have been established in various studies (Rahman et al., 2021). The total phenolic content of fresh garlic grown in the Gaziantep and Kastamonu regions was 65.12 mg GAE/100 g and 19.59 mg GAE/100 g, respectively. It was determined that the amount of total phenolic matter increased with the increase in the production period of black garlic produced from fresh garlic grown in both regions. In previous studies, it was reported that black garlic had higher total polyphenol content than fresh garlic. Total polyphenol content was reported to be 7-11 times higher than fresh garlic, and total phenolic acid content increased 4 to 8 times (Casas et al., 2017).

Table 2. Antioxidant activity and total phenolic content results

	DPPH (µmol Trolox /100 g)	ABTS (µmol Trolox /100 g)	TPC (mg GAE /100 g)
Gaziantep fresh garlic	53.66±4.06^{h}	188.14±9.98^{h}	65.12±1.71^{g}
G7	620±4.49^{f}	1081.85±39.47^{f}	165.43±1.03^{e}
G14	1292.38±41.31^{d}	2177.77±6.66^{d}	331.66±4.19^{c}
G21	2598.57±28.53^{c}	3770.74±93.07^{c}	586.81±1.52^{b}
G28	2929.52±42.24^{a}	4350±70.61^{a}	618.03±13.64^{b}
Kastamonu fresh garlic	56.85±2.66^{h}	109.55±0.86^{i}	19.59±0.93^{h}
K7	548.09±10.72^{g}	841.48±11.18^{g}	92.69±1.01^{f}
K14	1013.33±14.30^{e}	1655.92±16.32^{e}	211.51±2.02^{d}
K21	2631.42±29.51^{c}	3838.88±4.80^{c}	590.75±2.14^{b}
K28	2782.85±10.78^{b}	3970.74±10.01^{b}	624.24±9.58^{a}

$^{a-g}$Different exponential letters in the same column indicate a significant difference between the samples ($p<0.05$). G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 14 days, G21; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 28 days, K7; Black garlic produced from Kastamonu fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K21; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 28 days.

Table 3. Retention time and mass spectral properties of organosulfur compounds in garlic

Organosulfur compound	Retention time (min)	Abbreviation	Main ion	Fragment ion
(3R,5S)-5-methyl-1,4-thiazane-3-carboxylic acid	5,72	Cycloalliin	178.23	88.08, 91.04
(+)-S-allyl-l-cysteine sulfoxide	6,32	Alliin	178.15	88-74
(+)-S-(trans-1-propenyl)-l-cysteine sulfoxide	6,57	Isoalliin	178.2	88.00, 160.10
(+)-S-allil-l-sistein	10,64	SAC	162.1	145.1, 73.1
γ-l-glutamil-S-allil-l-sistein	17,38	GSAC	291.2	162,2, 144,8
γ-l-glutamil-S-(trans-1-propenil)- l-sistein	20,5	GSPC	291.2	201,1
γ-l-glutamil-phenylalanine	24,83	γGPA	295.3	178.0, 88.0
Allicin	55,3	Allicin	163.2	73.2, 41.1

Table 4. Organosulfur compound contents of garlic (mg/g)

	Gaziantep fresh garlic	G7	G14	G21	G28	Kastamonu fresh garlic	K7	K14	K21	K28
(+)-S-allil-l-sistein	38.31±0.01^{f}	175.35±2.01^{c}	198.33±0.01^{b}	215.98±0.87^{a}	166.81±0.43^{c}	57.28±0.64^{e}	185.95±0.71^{c}	220.67±0.95^{a}	112.80±0.47^{d}	173.30±0.75^{c}
(3R,5S)-5-methyl-1,4-thiazane-3-carboxylic acid	12.83±0.21^{e}	34.33±0.24^{b}	27.90±0.68^{c}	23.74±0.01^{d}	Nd	16.09±0.01^{d}	42.60±0.01^{a}	10.43±0.02^{f}	Nd	Nd
(+)-S-allyl-l-cysteine sulfoxide	5.03±0.02^{e}	39.05±0.72^{b}	42.59±0.31^{a}	26.235±0.40^{c}	Nd	3.43±0.01^{f}	24.22±0.01^{d}	Nd	Nd	Nd
(+)-S-(trans-1-propenyl)-l-cysteine sulfoxide	6.73±0.01^{d}	23.56±0.26^{b}	32.35±0.01^{a}	Nd	Nd	4.01±0.01^{e}	7.29±0.01^{c}	Nd	Nd	Nd
γ-l-glutamil-S-allil-l-sistein	4.40±0.01^{d}	7.62±0.01^{b}	Nd	Nd	Nd	9.98±0.01^{a}	5.47±0.01^{c}	Nd	Nd	Nd
γ-l-glutamil-S-(trans-1-propenil)-l-sistein	194.10±0.10^{a}	25.95±0.10^{g}	17.85±0.01^{h}	45.80±0.27^{d}	29.57±0.01^{f}	75.06±0.98^{b}	96.14±0.01^{c}	52.28±0.01^{d}	Nd	32.30±0.01^{e}
γ-l-glutamil-phenylalanine	9.64±0.01^{a}	6.20±0.10^{c}	Nd	Nd	Nd	9.40±0.01^{a}	8.28±0.01^{b}	Nd	Nd	Nd
Allicin	300.42±0.01^{a}	2.16±0.01^{c}	1.47±0.01^{d}	0.62±0.01^{f}	0.32±0.01^{h}	116.34±0.21^{b}	2.02±0.01^{c}	1.14±0.01^{e}	0.45±0.01^{g}	0.24±0.01^{i}

$^{a-h}$Different exponential letters in the same column indicate a significant difference between the samples ($p<0.05$). Nd: Not detected. G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 14 days, G21; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 28 days, K7; Black garlic produced from Kastamonu fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K21; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 28 days.

CONCLUSION

This study investigated the changes in the black garlic production processes of Kastamonu and Gaziantep garlic, which are Turkey's most produced garlic varieties. Within the scope of the study, samples were taken on the 7th, 14th, 21st, and 28th days of the black garlic production process at 65°C temperature and 70% humidity, and various analyses were performed. According to the results obtained, it was determined that total phenolic substances and antioxidant potentials increased in black garlic production processes of both regions compared to fresh garlic. In addition, it was observed that sucrose was the predominant protein in fresh garlic, while fructose was the predominant protein in black garlic. Regarding protein content, the ratios range from 5.8% to 7.3% in fresh garlic and were found to be around 13.1% to 14.2% in black garlic. As a result, it was determined that fresh and black garlic from the Gaziantep region had higher phenolic matter, antioxidant capacity, and sulfur compound contents. This study provides an important contribution to understanding the changes in the production processes of fresh garlic and the bioactive properties of black garlic obtained from garlic grown in the Kastamonu and Gaziantep regions.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare no competing, actual, potential, or perceived conflict of interest.

Author contribution

Hatice Kubra Sasmaz: conceptualization, data curation, formal analysis, funding acquisition, writing - first draft, writing - review & editing Hasim Kelebek: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing). Serkan Selli: Conceptualization, data curation, formal analysis, funding procurement, original draft writing, review, and editing. Turkan Uzlasir: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing).

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Structural analysis of cattle enterprises in Kayapınar district of Diyarbakır province

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Abstract

This research was conducted in person in Diyarbakir province, Kayapınar district, by means of a questionnaire with a total of 120 people engaged in cattle breeding in 21 settlements. The questionnaire addresses the existence of cattle farms, the general structural status of cattle farms, information provided by owners, feed supply and feeding practices, animal health and treatment practices, reproduction and artificial insemination practices, production and marketing of animal products on farms, housing status and support utilization status. It has been shown that the farms are small farms and 92% of the farms have closed barns. The fact that the majority of farm owners are between 40 and 61 years old can be seen as a danger signal for livestock. It was found that there is no cooperative in the villages except Cumhuriyet district, which brings the problem of marketing raw milk and dairy products. In the study area, it it has been observed that activities such as animal care feeding, estrus monitoring and application, and automation in animal husbandry are insufficient. For sustainable animal production, relevant public institutions and organizations need to work more intensively and carry out training, monitoring and evaluation activities to increase productivity per animal in enterprises, to train operators on herd management, care and feeding and animal health, and to meet expectations by solving problems in enterprises. This research provides us with important data about the general structure of cattle breeding in Kayapınar district of Diyarbakır province.

Keywords: Cattle enterprises, Diyarbakır, Kayapınar, structural analysis

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INTRODUCTION

Animal husbandry has always been of great social and economic importance in Turkey and has great potential for animal production due to its geographical and socio-economic conditions. Despite this potential, animal husbandry generally takes place as a secondary activity alongside crop production (Yılmaz and Koeknaroğlu, 2007; Baş Hozman and Akçay, 2016; Kösemen and Şeker, 2016).

Dairy cattle are the most important source of milk production. There is no short-term planning flexibility in dairy cattle activities due to reasons such as being a long-term production branch among livestock activities and the difficulty of converting the investments made into other investments (Şahin, 2001).

The importance of dairy farming in Diyarbakır extends to both social and economic aspects, contributing significantly to the livelihoods of the local population and the regional economy. Dairy farming is a major economic activity in Diyarbakır, providing income and employment opportunities for many individuals and families. The dairy sector contributes to the overall agricultural

economy of the region, generating revenue through the sale of milk and dairy products including milk, cheese, and yogurt, are essential components of the local diet, contributing to the food security and nutritional needs of the community.

Dairy farming is typically associated with rural areas, and its presence in Diyarbakır contributes to the overall development of rural communities. It helps sustain rural economies and prevents migration from rural to urban areas.

In Turkey, by the year 2022, there are a total of 16,8 m head of cattle consisting of 49% pure breed, 43% crossbreeds and 8% local breeds. According to TUIK data, cattle milk production in our country was 21.4 million tons in 2021. As a result of the studies on breeding, developments in animal nutrition and management have increased the carcass weight, which was 143 kg/head in 1991, to 285 kg/head in 2021. (TUIK, 2022)

Table 1. Number of Cattle and Buffalo in Türkiye, Diyarbakir and Kayapınar District

Species	Türkiye	Diyarbakır	Kayapınar
Cattle	17.692.655	559.883	24.063
Buffalo	182.717	17.195	489

Source: Türkvet, 2022

When the number of animals and milk production amounts according to the species are examined in Turkey; Approximately 35% of the existing cattle are milked and 90.5% of the milk produced is obtained from cattle. In general, dairy cattle enterprises in Turkey are seen as small family enterprises. When the studies on dairy cattle breeding are examined, it is seen that the high costs of the enterprises, the problem of feed production and supply, technical problems, and the problems encountered in marketing come to the fore. There are significant differences between regions in terms of cattle breeding in Turkey. Although there are studies in the literature that examine the characteristics of dairy farms in different provinces, it is considered important to repeat the studies, observe the changes in the farms and develop solutions to their problems.

This study aims to reveal the general structure of the cattle farms in the Kayapınar District of Diyarbakır province and offer suggestions for its solution.

Understanding the multifaceted importance of dairy farming in Diyarbakır is crucial for policymakers, researchers, and stakeholders to develop strategies that enhance the sector's sustainability, address challenges faced by farmers, and promote the overall well-being of the community.

Materials and Method

The study material was sourced from cattle enterprises situated in the Kayapınar district of Diyarbakır province through in-person questionnaire interviews.

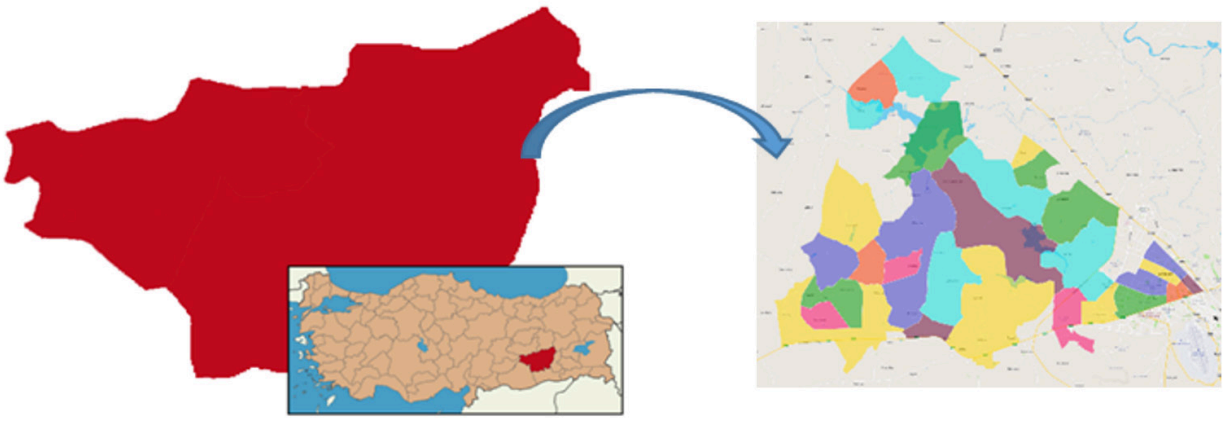


Figure 1. Study area location map

In the official Animal Recording and Data System (Türkvet) of the Ministry of Agriculture and Forestry in Kayapınar district, it has been determined that there are a total of 933 active holdings and 22144 cattle in 2022.

In this population size, the number of samples to be taken for a study to be carried out with 10% sampling error and 95% confidence level was calculated as 120 enterprises using the formula given below (Yazıcıoğlu and Erdoğan, 2004).

$n=N.t^2.p.q/d^2(N-1)+t^2.p.q$

n : Sample size
N : Population size (453)
t : t scale value at 95% confidence interval (1.96)
p : 0.5 (50% incidence)
q : 0.5 (50% incidence)
d : Sampling error (0.10)

Population Size (N)

The total number of active holdings and cattle in the Kayapınar district, as recorded in the official Animal Recording and Data System (Türkvet), is identified as the population size. In this case, N is 22144 cattle distributed among 933 active holdings.

Confidence Level (95%)

The confidence level represents the likelihood that the true population parameter falls within the calculated confidence interval. A 95% confidence level is commonly used in statistical studies, and it corresponds to a t scale value of 1.96. This value is derived from statistical tables and represents the standard deviation multiplier for a normal distribution.

Sampling Error (10%)

The sampling error, denoted as 'd,' signifies the acceptable margin of error in the estimate of the population parameter. In this study, a 10% sampling error is specified, meaning that the researchers aim for the estimated value to be within 10% of the true population parameter.

Incidence (p and q)

The incidence, represented by 'p' and 'q,' refers to the proportion of the population exhibiting the characteristic of interest. In this case, since the incidence is not explicitly known, it is assumed to be 50%, resulting in p = q = 0.5. This assumption is conservative and results in the maximum required sample size for a given confidence level and sampling error.

The primary data source for this research emanates from in-depth, face-to-face surveys conducted with 120 enterprise owners. The surveys were administered across 21 villages within the Kayapınar District of Diyarbakır during the months of July and August in 2023. The study focuses on breeders residing in the selected villages, forming the core basis of the research data.

The face-to-face survey approach ensures a direct and personalized interaction with enterprise owners, allowing for a comprehensive understanding of their perspectives and experiences in the dairy farming sector. The choice of villages and the sampling process provide a representative cross-section of the local community involved in dairy farming, contributing to the robustness and reliability of the research findings.

The data collected through the questionnaire were entered into the Excel program after the necessary coding and controls were made. While evaluating the data, simple averages and percentage calculations were used. A questionnaire was prepared to determine the general characteristics and practises in enterprises. The answers given by the business owners during the visits were recorded by entering this survey form, and the details about the business were noted by making observations during the visit. The statistical analysis of the data obtained from the research results was conducted using the SPSS 15.0 software package, employing frequency analysis

RESULTS AND DISCUSSION

Socio-Demographic Characteristics of Households

According to the study, 3.3% of cattle enterprises owners age are 18-30 years old, 9.16% are 31-40 years old, 79.1% are 41-60 years old and 8.3% are 61 years old (Figure 2).

The findings from the data reveal a notable demographic trend in livestock activities in the province, indicating a predominant involvement of middle-aged individuals. The aging trend observed over the years is particularly evident in the concentration of livestock activity owners within the 41-60 age range. This demographic shift aligns with similar studies conducted in different regions. For instance, Kaygısız and Özkan (2021) study in the Tekkeköy district of Samsun province reported that 62.50% of breeders fell within the 40-60 age range. Similarly, Özdemir et al. (2021)

found an average age of 43.56 years among breeders in the Balıkesir Gönen district. Tutkun et al. (2017) study in Diyarbakır Province reported a 48% ratio of breeders aged 41-60. In a study conducted in the Viranşehir district, it was determined that the average age of breeders is 41.9 years (Delebe and Yazgan, 2023). In another study conducted in Iğdır province, it was also determined that the average age of breeders is 44.9 (Yılmaz et al. 2020).

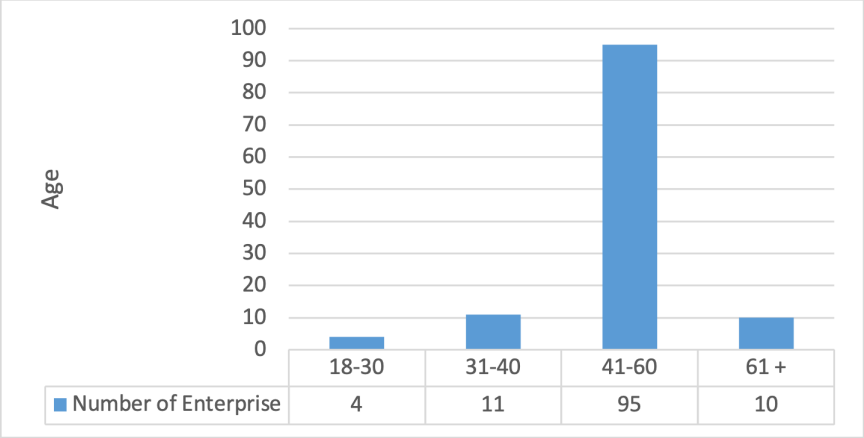


Figure 2. Distribution of breeders by age

Remarkably, in the current study, the age distribution stands at approximately 79.1%, indicating a substantial increase when compared to a previous study in the same province. This suggests a significant shift in the age composition of individuals engaged in livestock activities, with the breeder's age ratio nearly doubling.

A noteworthy concern is the limited involvement of youth in animal production, with only 3.3% falling within the 18-30 age range. This may have implications for the sustainability and succession of livestock activities in the long term.

It has been observed that, similar to other regions in Turkey, individuals engaged in cattle farming are mostly in the middle age group, and there is less interest from the younger population in cattle farming.

Additionally, literacy rates among breeders in animal husbandry present a diverse picture, with 42% being illiterate, 20% completing primary school, 24% secondary school, and 11% reaching high school. When the education levels of dairy farming business owners are evaluated according to scales, it is determined that, for small enterprises, approximately 49.1% are primary school graduates, for medium-sized enterprises, around 45.4% have primary school education, and for large enterprises, approximately 5.5% are primary school graduates. Across all scales, it is reported that high school graduates, with 49.6%, constitute the numerical majority (Mat &Cevger, 2020).

These findings underscore the importance of considering both demographic and educational factors in shaping future policies and interventions aimed at sustaining and advancing animal husbandry practices in the region (Figure 3).

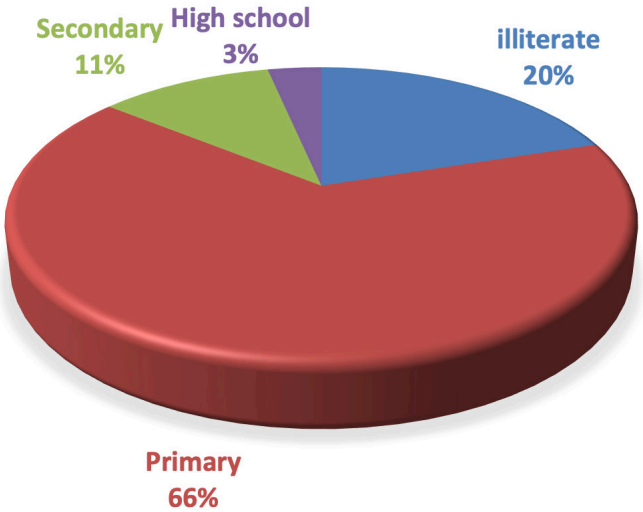


Figure 3. Literacy rate of enterprice owners

It has been determined that as the literacy rate increases, the number of people engaged in animal husbandry decreases.

Animal presence of enterprises

The study encompasses enterprises with a total of 2987 cattle distributed across various age groups.There are a total of 1541 (51,5%) cows, 517 (17,3%) heifers, 505 (16,9%) bullock, 412 (13,8%) calves and 12 (0,4%) bulls in the holdings (Figure 4).

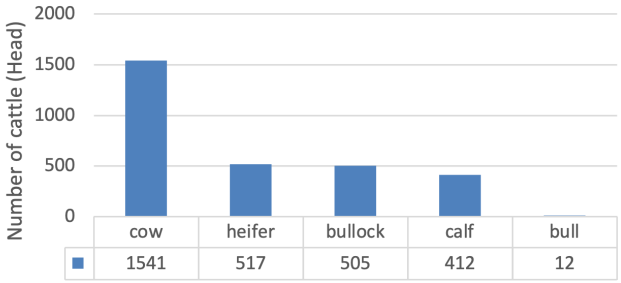


Figure 4. The animal presence

According to the research findings, 60% of the cattle are cross-breed, 23% are pure breeds and 17% are local breeds (Figure 5). Most farms enterprises prefer crossbred cattle due to insufficient barn and maintenance-feeding conditions. In a study conducted by Bakan and Aydın (2016) in Ağrı the province, they found the local breed rate is 11.6%. Tugay and Bakır (2008) found pure, cross-breed and local breeds as 5.3%, 23.6% and 71.1%, respectively in Giresun province.

In the study conducted in Balikesir Province, it was observed that 87.1% of the enterprises within the research scope preferred dairy breeds, while 12.9% opted for dual-purpose breeds (Mat & Cevger). A study conducted in Kahramanmaraş revealed that 61.79% preferred dairy breeds and 38.21% preferred dual-purpose breeds. Similarly, in a research study in the Thrace region, the preference was 80% for dairy breeds and 20% for mixed breeds (Güzel and Aybek, 2017; Keskin and Dellal, 2011). Regarding producers, it is believed that dairy cultural breeds have a superior breeding value, milk yield, and ease of calving compared to mixed breeds. On the other hand, mixed-breed animals are considered to have higher values in terms of slaughter value, economic lifespan, and lactation duration (Topçu, 2008).

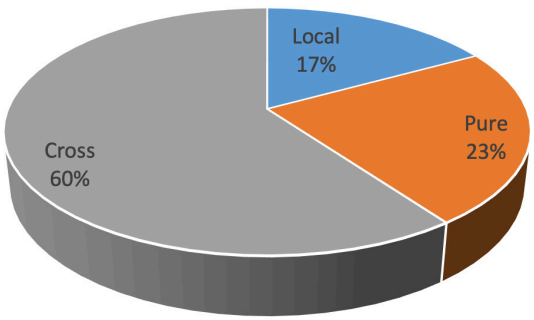


Figure 5. Cattle breed distributions

Cattle barns types

In this study, data was gathered through face-to-face surveys involving 120 enterprise owners. The distribution of barn types within these enterprises revealed that 91.6% were closed, 5.0% were semi-open, and 3.4% were open (Figure 6). A comparative analysis with a study conducted by Yılmaz et al (2020) in Iğdır province indicates notable differences. In the Iğdır study, 88.3% of barns were closed, with 8.0% being semi-open and 3.7% open. The findings from the current research surpass these percentages, signifying a higher prevalence of closed barns, semi-open barns, and open barns.

When examining some studies conducted in different provinces, it has been reported that existing businesses in Kayseri province prefer tied-stall barn types, with 75.0% (Şahin, 2009), in Çankırı province, 73.9% (Yıldız, 2013), in Sakarya province's Hendek district, 43.8% (Karaca, 2020), and in Austria, 40.2% of the businesses prefer tied-stall barn types (Klein-Jobstl et al., 2015).

Furthermore, when juxtaposed with the study by Özsağlıcak and Yanar (2022), which focused on cattle enterprises, a distinct pattern emerges. In the present study, 95.0% of cattle enterprises reported closed barns, while 4.8% and 0.3% had semi-open and open barns, respectively. This variation in barn types among cattle enterprises highlights the nuanced preferences and practices within different regions or populations. The results suggest a higher inclination towards closed barns in the current investigation compared to the findings of Özsağlıcak and Yanar's study. These disparities could be attributed to various factors, including geographical, climatic, or management differences that influence the choices of barn types among livestock owners.

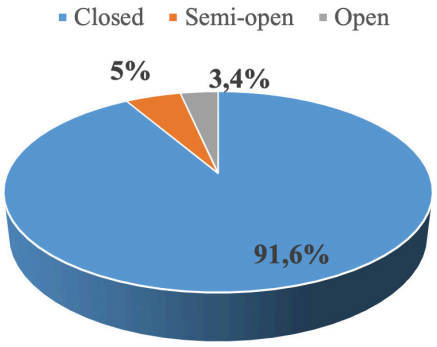


Figure 6. Barn types

Demir et al. (2014) in a study conducted in Kars province, 95.1% of the barns are closed and 4.9% are semi-open barns and 83.1% of closed barns The vast majority of them are of fixed-attached barn type. In another study, % 97 of barns are closed and 3% are open (Kaygısız and Tümer, 2009) while Yaylak et al. (2015), in their study in Ödemiş district of İzmir province, 8.7% of the barns were closed, 15.2% semi-closed and 76.1% were open

Cattle barn capacity

The average capacity of the barns was determined as 28 cattle. In the study, barn capacities vary, 6% of the enterprises have a capacity of 1-5, 14% of 6-10, 42% of 11-20 and 18% of 21-50, and barns with a capacity of 51 and more was not observed (Figure 7).

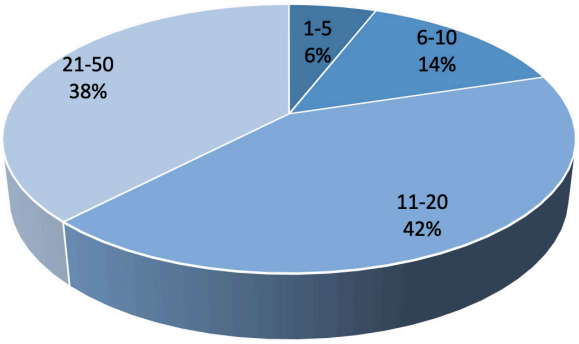


Figure 7. Barn capacity of the owners

The majority of enterprises opt for stone in constructing barn walls, concrete for barn floors, sheet metal for barn roofs, and wood for feeders. According to a report by Kaygısız and Tümer in 2009, briquettes are employed as a heating source in 40.0% of the existing cattle barns in Kahramanmaraş province.

In a separate study conducted by Mundan et al. in 2018 in Şanlıurfa province, it was documented that the wall-building material 69.7% briquette, 11.9% stone and 18.4% bricks were used. It has been determined that it is the most common occurrence in cattle barns in Giresun province, the stone material at 62.5%, then briquette at 27.9%, wood at 8.6% and adobe at 1.1% (Tugay and Bakır, 2006).

Differences between regions, climatic, and economic levels and growers' differences between provinces occurred due to preferences and habits arising from socio-demographic differences.

Animal feeding

For economical and rational animal feeding, it is necessary to take into account the genetic characteristics of the animals as well as their productivity, physiological state (pregnancy), health status and environmental conditions.

Within the scope of the research, it was found that 22% of the examined existing enterprises did not provide animal feed based on different ages and genders. Animal feeding was implemented with the same content and amount while 41% of the businesses stated that grouped and 37% stated that they partially implemented (Figure 8)

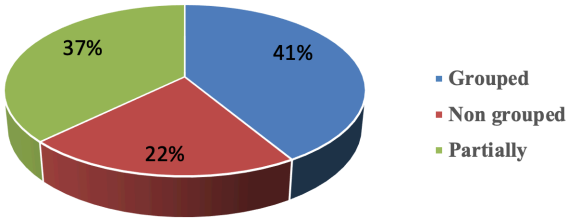
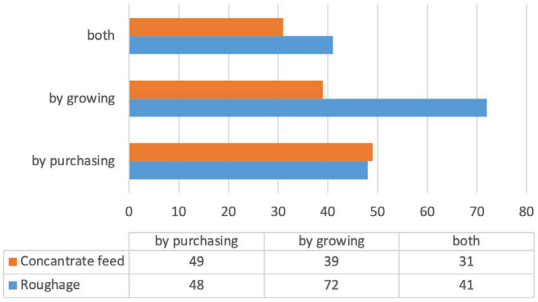


Figure. 8. Animal feeding methods

In the study, animal feeding practises was presented in Figure 9. In the research, 43.3% of the enterprises stated that they procured by purchasing the roughage, 22.5% of them by groving and rest of them 34.1% declared that they both by growing and puchasing to meet requirments of the enterprises



	by purchasing	by growing	both
Concantrate feed	49	39	31
Roughage	48	72	41

Figure 9. Concentrate and roughage procure situations of the enterprises

It was found that 41% of the enterprises acquired concentrated feed through a dealer, 33% fed their animals with the grains they grew, and 26% of both by purchasing and growing. The most important problem of farms is the high forage and concentrate feed prices.

They stated that most of the existing enterprises visited within the scope of the research had difficulties in supplying feed. It has been observed that the enterprises which stated that they did not have difficulties in supplying feed, also carried out plant production activities and used products such as wheat, barley, corn straw and straw obtained from them as animal feed.

In addition, the rate of growing forage crops and silage corn in farms is also low. It was observed that 40% of the breeders within the scope of the research used silage in animal feeding, while 60% did not use silage and did not even know what silage was.

Reproduction

Reproduction is considered the basis of animal breeding and is an indispensable condition for continuity in animal production. The main goal in dairy cattle enterprises is to obtain one offspring per year from each cow. Necessary conditions must be created for healthy reproduction to occur in enterprises. For healthy reproduction and fertility, animals must be fed well and balanced, preventive medical measures must be taken, and shelter and care conditions must be improved. In addition to the factors listed above, for healthy reproduction and successful fertility in cattle farms, it is of great importance to follow the estrus cycle of the animals well and perform timely breeding or artificial insemination. Within the scope of our research, the most common reproductive problems in enterprises, as well as estrus monitoring and breeding methods used (natural and artificial insemination) were examined. In the research, it was determined that 69% of the breeders observe estrus (heat) and 31% did not (Figure 10).

When the study examined which method breeders preferred in Insemination, It was determined that while almost all of the enterprises (94%) preferred natural insemination by using sire instead of artificial insemination, 6% of the enterprises applied the artificial method only (Figure 11).

In a study conducted in Giresun province by Tugay and Bakır (2011) reported that 38.9% of the enterprises performed artificial insemination and 58.2% performed natural insemination. The rate obtained in this study was found to be

higher than performing artificial insemination.

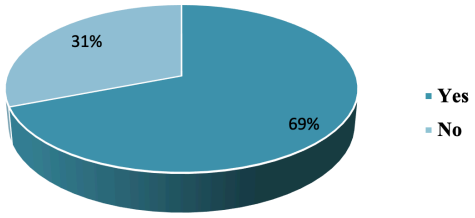


Figure 10. Estrus observation

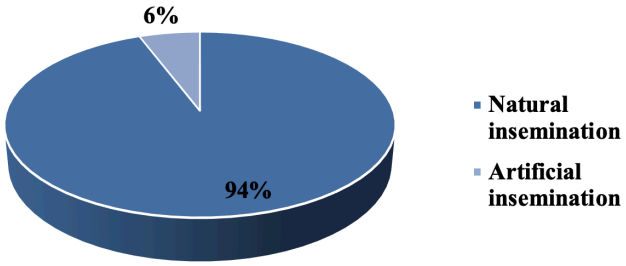


Figure 11. Insemination methods used by breeders

In the study, the answers given by the breeders within the scope of the research regarding the time intervals in which they inseminated the cows after they came into heat. Accordingly, 46% of the breeders declared that they had it done in the first 0-4 hours after the heat was observed, 40% of them after 5-10 hours and the rest of 14% was 11-18 hours (Figure 12). Traditionally, the optimal timing for artificial insemination (AI) in cattle has been conventionally linked to the onset of estrus, occurring within 6 to 24 hours from the initial signs of estrus. Nevertheless, recent research indicates a shift in this interval, proposing that AI should be conducted within 16 to 6 hours before ovulation, thereby aligning it more closely with the conclusion of the estrus period.

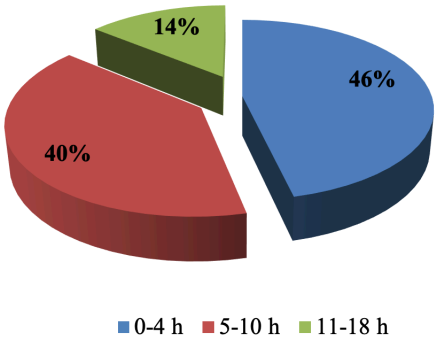


Figure 12. İnsemination timing

Milking

In cattle farms, ensuring the health of udders and maintaining hygiene during the milking process are crucial factors influencing the quality and dependability of the obtained milk. The way and conditions of storage of milk after milking are other important factors regarding the safety of milk. It has been determined that milking is generally done twice a day (99%) in farms, in the morning and evening, and in a very small number of farms (1%) in large capacity modern enterprises milking is done 3 times a day (Figure 13)

Cows are typically subjected to two milking sessions per day, although the frequency may vary based on the farmer's management strategy. The most prevalent approach involves milking at least twice a day, ensuring the comfort of the cows as their udders have a limited capacity to hold milk before requiring expulsion.

It has been found in a study conducted in Erzincan by Sağalcık and Yanar (2022) that The rate of enterprises where

milking is done once a day is 1.4% and done twice is 98.6%

It was determined that 86% of the milking was done by hand and 14% of the milking was done by machine in a village where there was only a cooperative (Figure 14). Aksoy et al. (2014), in a study conducted in Erzurum province, found the rate of enterprises milking by hand as 88.7%. Koçyiğit et al. (2016) found that 85% of milking by hand in a study conducted in Hınıs district of Erzurum province.

In a study conducted in Erzincan province, it was reported that in 47.4% of the enterprises, milking is done manually, in 47.1%, mobile milking machines are used, and in 5.5%, milking is carried out in milking units (Özsağlıcak & Yanar, 2022).

A study conducted in the Tekkeköy district of Samsun province, ıt was found that in 31% of the enterprises, milking is done manually and in 69% of made by milking machines (Kaygısız and Özkan,2021)

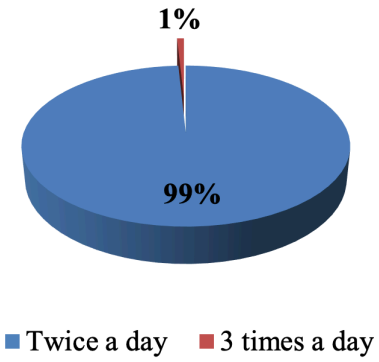


Figure 13. Milking time

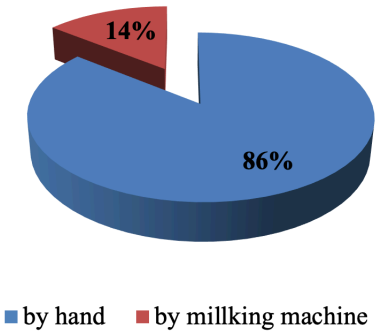


Figure 14. Milking methods

Within the scope of the research, 90% of the breeders pay attention to milking hygiene including the pre- and post milking routines as well as the cleanliness of the equipment used to milk the cows. Premilking procedures such as dipping, dry wiping, fore stripping, and cleaning or drying of the teats and teat ends.

Within the surveyed enterprises, the study revealed that 47.4% employed manual milking techniques, 47.1% utilized mobile milking machines, and 5.5% conducted milking in designated milking parlors. Furthermore, a significant 94.1% of breeders practiced udder cleaning and disinfection both before and after the milking process. Notably, the research found that only 47.7% of the enterprises underwent regular checks for mastitis. (Özsağlıcak and Yanar,2022)

Marketing

A small proportion of this milk (1%) was marketed as raw milk only and the rest of the milk in the enterprises was sold after processing into different products such as 48% yoghurt, 45% cheese, and 6% butter (Figure 15).

In the Giresun region, dairy farming businesses evaluate their milk as butter, yogurt, cheese, raw milk, and curd, as reported by Tugay (2007). In another study conducted in Ağrı province, 15.1% of the operators reported selling their produced milk to milk collectors, 2.8% to dairies, and 82.1% reported using it in other ways (for household needs, selling to interested parties, etc.) Bakan (2014).

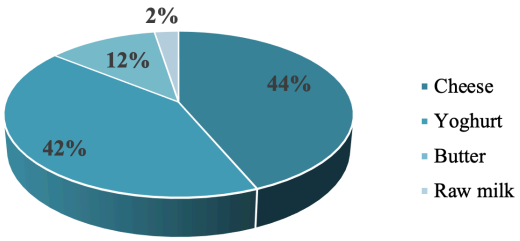


Figure 15. Raw milk marketing methods of the enterprises

Animal Health and Veterinary services

The study outcomes indicate that nearly all enterprises (99.2%) prioritize protective vaccination. Veterinary services are sought by a substantial 90.9% of the enterprises, with 70.4% seeking veterinary care only when a disease is observed, and 29.6% opting for regular veterinary check-ups. Common animal diseases seen were declared as brucellosis, mastitis, foot and mouth disease (FMT), theileria and septicemia (Figure 16). A study conducted by Bakır and Kibar (2019) in Muş province, determined the incidence of mastitis (64.7%) in cows raised in enterprises is higher than in this study (5,8%).

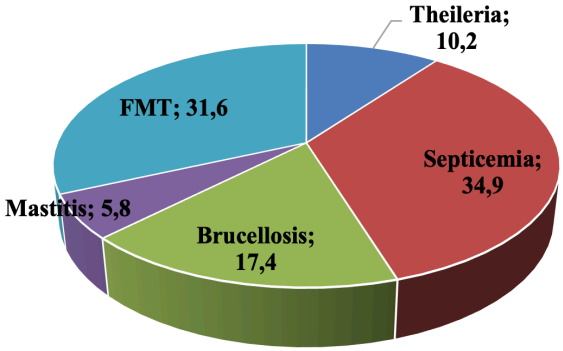


Figure 16. The most common animal diseases

One of the most effective health protection methods for animals is vaccination. Vaccinating on time and correctly can minimize the risk of disease in animals. The quality of the vaccines used, cold chain application, the vaccination schedule and the method of administering are important factors affecting the disease-preventive effectiveness of the vaccine. It has been determined that failure to vaccinate on time causes widespread epidemic diseases, especially FMD, and serious economic losses.

CONCLUSION

The majority of the cattle farms under investigation are small enterprises. This situation means that they cannot specialize in animal production and prevents them from increasing production. As far as animal diseases are concerned, foot and mouth disease and mastitis are particularly prevalent in the district. It was found that vaccinations and other health protection measures are not effectively implemented on the farms. Cattle farms in Diyarbakır suffer from high input costs for production. It was determined that the type of organization in the cooperative structure is not very common in cattle farms and there is only one cooperative in the district. For sustainable livestock production, the relevant public institutions and organizations need to work more intensively and carry out training, monitoring and evaluation activities to increase productivity per animal on farms, train operators in herd management.

To ensure sustainable animal production in the Kayapınar district, it is imperative for relevant public institutions and organizations to intensify their efforts. This involves conducting comprehensive training, monitoring, and evaluation activities aimed at increasing productivity per animal. Operator training on herd management, care, feeding, and animal health is essential. Addressing existing challenges within enterprises will contribute to meeting expectations and fostering a more sustainable and efficient cattle breeding sector in the region. Overall, this research serves as a valuable resource, providing essential data for understanding and addressing the general structure of cattle breeding in the Kayapınar district of Diyarbakır province.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

This study was approved by Dicle University Ethics Committee, Diyarbakir, Türkiye (Approval No: 624869, Date: December 26, 2023).

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Data availability

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Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Heavy metals in tailings and soils in the Pb-Zn mining areas of North-west Türkiye and health risk evaluations

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Abstract

Improper mining waste and tailing management in Pb-Zn mining areas (Balya and Koru) in the north-west Türkiye have not been researched sufficiently. Accordingly, concentrations of heavy metal were determined in mine tailing and soils taken from Balya and Koru, and a health risk evaluation caused by heavy metals was performed. Average Cd, Cr, Cu, Mn, Ni, Pb, and Zn concentrations in mine tailings in Balya are 35.2, 17.8, 354.7, 1735, 10, 10089, 3730 mg kg^{-1} and these values were determined as 9.9, 8.9, 101.5, 1308, 4.5, 1871, 1375 mg kg^{-1} in the tailings in Koru, respectively. The concentrations of heavy metals in the soil samples taken from both Balya and Koru were determined to be lower. The evaluation of heavy metals' health risks was performed according to both non-carcinogenic and carcinogenic effects. The primary route of heavy metals in adults and children has been determined by oral intake. For both children and adults, the order of the carcinogenic effects of heavy metals in mine tailings and soils in Balya and Koru was Cd > Pb > Ni > Cr. As the carcinogenic risk values of Cd and Pb for adults and children in mine tailing and soils in Balya were above the limit value, the children's Cd carcinogenic risk values were found above the limit value in mine tailing and soils in Koru. The mining area in both Balya and Koru poses a risk to human health since it is close to settlements.

Keywords: Heavy metals, Lead–zinc mine, Soil pollution, Health risk assessment, Türkiye

INTRODUCTION

Human activities including agricultural activities, vehicular emissions, inappropriate waste removal and disposal, fossil fuels and mining facilities generate serious damage on environmental matrices (soil, plant, water and sediment). Mining activities constitute the most striking and the primary source of heavy metals in receiving bodies (Azhari et al. 2017; Nassiri et al. 2021). Mine wastes such as tailing, soil and dust result in water, air and soil pollution, loss of biodiversity and various health problems for people living around mining facilities (Du et al., 2019; Tran et al., 2022).

Exposure of human body to excessive concentrations of heavy metals can damage nervous system, cause kidney dysfunction, hypertension, cancer and increase the risk of fetal death (Kapwata et al., 2020; Zhao et al., 2019). Heavy metals are taken into human body through ingestion, inhalation or dermally (Botsou et al., 2020; Kan et al., 2021; Parlak et al., 2022, 2023). Qi et al. (2016) conducted a health risk assessment study for children exposed to soil heavy metals in a Pb-Zn mine district of Yunnan Province of south-west China. It was found that long-term Pb/Zn mining activities caused serious soil pollution. It was

also determined that children were exposed to greater health risks than adults and the highest hazard index was encountered in Pb (57-74%). Jahromi et al. (2020) calculated health index values for heavy metals (Cd, Pb, and Zn) of the soil samples taken from the nearest mining area of Isfahan (Iran) and determined that all health index values were higher than 1, indicating negative impacts on human health.

In recent years, number of studies on Pb-Zn mining areas of different countries such as Nigeria (Adewumi et al., 2021), Algeria (Arab et al., 2021), China (Cao et al., 2022), Spain (Garcia-Lorenzo et al., 2019) and Iran (Tehrani et al., 2023) has increased. There are also previous studies on heavy metals in Pb-Zn mining regions of Türkiye (Hanilçi and Öztürk, 2011; Koz, 2014; Çelebi and Öncel, 2016; Çiçek and Oyman, 2016; Hanilçi et al., 2019), but none of them focused on potential health risks of heavy metals. Therefore, this study will be the first comprehensive study in Pb-Zn mining areas of north-west Türkiye. Objectives of the present study was set as to assess spatial distribution and concentrations of heavy metals in tailings and soils of lead-zinc mining area in Balya (Balıkesir) and Koru (Çanakkale) districts; to evaluate possible sources of contamination in tailings and soils in Pb-Zn mining areas using principal component analysis and to evaluate the possible impacts of heavy metals in tailing and soils of the research areas on human health (adults and children).

MATERIALS AND METHODS

Study Area

Balya-Balıkesir Pb-Zn Mine: Balya is located in the Southern Marmara part of the Marmara Region, within the borders of Balıkesir province. It is surrounded by Manyas and Gönen in the north, Central district of Balıkesir in the east, İvrindi in the south, Yenice and Havran districts in the west. Balya is located between 27°20' - 27°50' east longitudes and 39°35' - 39°55' north latitudes (Figure 1). More or less 70% of Balya consists of mountainous areas, the rest consists of rough terrains. At 225 meters above sea level, Balya has hot, dry summers and cool, rainy winters. According to the long-term average (1938-2020), the average temperature of Balya is 14.7 ºC and the monthly total precipitation is 524 mm (GDM, 2022). There are Paleozoic, Mesozoic and Tertiary formations in the study area. There are mainly Permian-aged (Balya Formation) allochthonous limestones. A series of Triassic-aged (Karakaya Formation) claystone, sandstone, limestone and pebble stones unconformably are located on the parent rock (Budakoğlu and Pratt, 2005). The distance of mining site to Balya district is 1.6 km. Balya mining site lies between the villages of Patlak and Çakallar (Figure 1). Oak (Quercus) is the primary tree species in Balya. Red pine (*Pinus nigra*) species are encountered in the south and southwest of the mountains and there are also black pine (*Pinus nigra*) trees at higher altitudes. Hungarian oaks (*Quercus frainetto*) and Turkish oaks (*Quercus cerris*) are observed at relatively high altitudes and in the plateaus extending up to 600 m. These species have the appearance of clustered shrubs in areas with high level of destruction. Maquis lands have expanded their borders as a result of the destructions in the area where the primary vegetation is dry forest due to climate conditions. Maquis species include mock privet (*Phillyrea latifolia*), Greek strawberry tree (*Arbutus andrachne* L.), strawberry tree (*Arbutus unedo* L.), juniper (*Juniperus oxycedrus*) and Spanish broom (*Spartium junceum*) (Öncel, 2016). Balya mining site is an uncontrolled dumping site that has been in direct interaction with the receiving environment for many years (Figure 2A). The tailings interact with surface waters (rain water, Sarısu/Maden Stream, Kocaçay, Madra, Manyas Dam Reservoirs), groundwater, soil through dusting and therefore with humans and living things. Amount of tailing at Balya mining site is estimated to be between 1.000 - 2.355 million tons (Aka, 2020). Balya tailings contain pyrite (FeS_2), sphalerite (ZnS), galena (PbS), and chalcopyrite ($CuFeS_2$) minerals (Budakoğlu and Pratt, 2005).

Balya Pb-Zn mine is a mine known to exist since ancient times and operated by primitive ways. In the modern sense, it was first started to be operated by a French company in the 1880s. It was operated by the French company at intervals until 1939. The mine was closed in 1940 because the production revenues did not cover the operating costs (Arslan, 2010). The Pb-Zn mine was reactivated in 2005 and two private companies obtained the license to operate it. It is still the largest and oldest Pb-Zn deposit discovered in Türkiye (Çelik Balcı et al., 2014).

Koru (Lapseki-Çanakkale) Pb-Zn Mine: It is located in the north-west of Türkiye, on the Biga Peninsula, within the borders of Çanakkale province and in Korukoy area of Lapseki district (Figure 1). The geology of Biga Peninsula generally consists of metamorphic assemblages, ophiolitic rocks, overlying Neogene basin sediments and the products of magmatic activity that started from the Eocene and continued until the Pliocene (Bozkaya et al., 2020). Lapseki district has a typical Mediterranean climate with a high precipitation density in autumn and spring, cold in winters, hot in summers and air movements throughout the year (Türkeş, 1996). According to long-term average (1938-2020), the average temperature of Koru is 15.6 ºC and the monthly total precipitation is 620 mm (GDM, 2022). The distance of Koru mining site to the nearest settlement, Asmalı village, is 2.9 km. Koru mining site is located between the villages of Asmalı, Eskikışla and Karaömerler (Figure 1). The distance between Koru mining site and the village of Eskikışla and Karaömerler is 3.3 km and 4.3 km, respectively. In Lapseki, natural vegetation consists of a few maquis species of christ's thorn (*Paliurus spina-christi*), Greek strawberry tree (*Arbutus andrachne* L.), kermes oak (*Quercus coccifera*),

erica (*Erica* L.), mock privet (*Phillyrea latifolia*), rockrose (*Cistus* spp.), strawberry tree (*Arbutus unedo* L.), Spanish broom (*Spartium junceum*), cotoneaster (*Cotoneaster*), rhododendron (*Rhododendron*), wild blackthorn (*Prunus spinosa*), blackberry (*Rubus fruticosus*), wild olive (*Olea aleaster*), sumac (*Rhus coriaria* L.), styrax tree (*Styrax officinalis* L.) and red pine (*Pinus brutia*) (Koca, 2003). The tailings are discharged into receiving environments and there is no data on the amount of tailing for Koru mining site (Figure 2B). Koru tailings contain galena, sphalerite (ZnS), chalcopyrite, bornite (Cu_5FeS_4), and tennantite ($Cu_{12}As_4S_{13}$) minerals (Çiçek and Oyman, 2016).

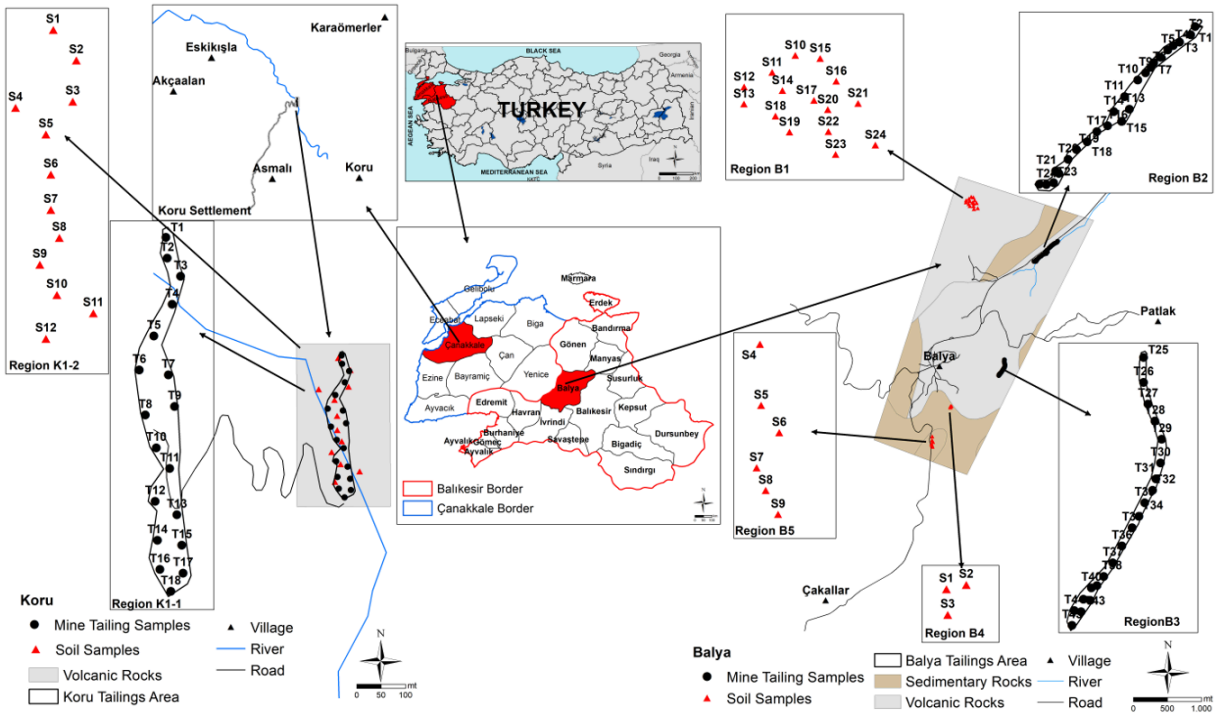

Figure 1. The location of the case areas and the places where mine waste and soil samples were taken

Figure 2. A. Tailings in the Balya mining area, B. Tailings in the Koru mining area

Sampling, Soil and Tailing Analysis

Soil and tailing samples were taken from the Pb-Zn mining regions Balya and Koru between April 2018 and May 2019 with the use of random sampling method. Totally, 45 tailing and 24 soil samples were taken from 5 different regions of Balya and 18 tailing and 12 soil samples were taken from 1 region of Koru mining site. About 1 kg sample was taken from 0-20 cm depth. Each sample was divided into 3 sub-samples. Soil samples were taken from close vicinity of mining areas. Especially for Koru mining area, soil and tailing sampling points were quite close to each other. Coordinates of all sampling points were determined with a Global Positioning System (GPS).

Tailing and soil samples were then to laboratory, air dried, large particles were broken into small pieces with a wooden hammer and passed through 2 mm sieve. Some physicochemical analyses were performed on these samples. For soil texture (fine sand, coarse sand, silt and clay; %), Bouyoucos Hydrometer method was used (Gee and Or, 2002). Soil EC (dS m^{-1}) and pH values were measured from saturation paste extract and lime content (%) was determined in accordance with the procedures outlined in Burt (2004). Organic matter (OM) was determined in accordance with Nelson and Sommers (1996).

For heavy metal analyses (Cd, Cr, Cu, Mn, Ni, Pb, and Zn), samples were passed through 100 µm sieve and subjected to acid-digestion in aqua regia (HNO_3:HCl mixture, 1:3 ratio) (USEPA, 1996). Heavy metal concentrations of tailing and soil samples were measured in an ICP-OES device (Varian 710-ES model). Method validity was tested with the use of a verified reference material (NIM-GBW07425, soil). Percent recoveries are provided in Table 1. Recoveries varied between 88 - 104% and the findings were acceptable.

Table 1. Certified and measured values (in mg kg^{-1}) and recovery rate (%) of heavy metals of various standard reference materials (NIM-GBW07425, soil)

Metals	Certified values	Measured values	Recovery(%)
Cd	0.13±0.01	0.14±0.01	95.2±4.1
Cr	59±3	62.6±2.5	94.3±3.8
Cu	21.4±1.2	24.3±1.5	88.2±5.4
Mn	572±14	553.3±22.5	103.8±4.2
Ni	25.4±1.3	27.3±0.6	92.9±2.1
Pb	24.7±1.4	24.2±0.9	102.3±3.6
Zn	65±5	67.6±4.5	96.3±6.5

Health Risk Evaluation

Adults and children are walking around mining sites, children are also playing and livestocks are grazing on mining sites. In addition, people collect fruit from the shrubs and trees around the mining site and also collect edible wild plants. Therefore, health risk assessment of heavy metals was also performed in this study. Non-carcinogenic and carcinogenic health risks have been estimated for adults and children exposed to tailing and soil heavy metals (USEPA 1997, 2011)**.** Several researchers stated the most important potential exposure pathways as ingestion and dermal pathway (Li et al. 2014; Jahromi et al. 2020). Therefore, ingestion and dermal pathways were used in present study to determine adverse health effects. The average daily dose ($ADD_{ingestion}$ and ADD_{dermal}) was calculated with the use of the following equation (USEPA, 1989, 1997, 2011, 2019).

$ADD_{ingestion}$ (mg $kg^{-1}d^{-1}$) =Cx (IngRxEFx ED)/BWxAT xCF (1)

ADD_{dermal} (mg $kg^{-1}d^{-1}$) =Cx SAxAFxABSxEFxED/BWxAT xCF (2)

The parameters used to calculate the average daily dose ($ADD_{ingestion}$ and ADD_{dermal}) are shown in Table 2.

Table 2. Exposure factors of heavy metals (in mg kg^{-1} d^{-1}) used in this study

Parameters	Symbols	Units	Values	References
Heavy metal concentration	C	mg^{-1} kg^{-1}	Present value	-
Ingestion rate-adult	$IngR_a$	mg^{-1} day^{-1}	20	USEPA, 1997
Ingestion rate-child	$IngR_c$	mg^{-1} day^{-1}	200	USEPA, 1997
Skin surface available for exposure-adult	SA_a	cm^2	6032	USEPA, 2004
Skin surface available for exposure-child	SA_c	cm^2	2800	USEPA, 2004
Skin adherence factor-adult	AF_a	mg cm^{-2}	0.07	USEPA, 2004
Skin adherence factor-child	AF_c	mg cm^{-2}	0.2	USEPA, 2004
Dermal absorbsion factor	ABS	unitless	0.001	De Miguel et al., 2007; Botsou et al.,2020

Exposure frequency-adult	EF_a	days year^{-1}	350	USEPA, 2011
Exposure frequency-child	EF_c	days year^{-1}	104	USEPA, 2011
Exposure duration-adult	ED_a	year	20	De Miguel et al., 2007; Botsou et al.,2020
Exposure duration-child	ED_c	year	6	De Miguel et al., 2007; Botsou et al.,2020
Conservation factor	CF	kg mg^{-1}	10^{-6}	USEPA, 2011
Body weight-adult	BW_a	kg	70	USEPA, 2011
Body weight-child	BW_c	kg	15	USEPA, 2011
Average time for non-carcinogenic effects-adult	AT_a	day	7300	USEPA, 1989
Average time for non-carcinogenic effects-child	AT_c	day	2190	USEPA, 1989

Potential health risks for both adults and children who were exposed to heavy metal- contaminated soils were assessed through hazard quotient, calculated with the use of Eq. 3:

Hazard Quotient (HQ)=ADD/RfDs (3)

In equation, RfD_s is reference dose for heavy metals, which is provided in Table 3.

Table 3. Values of RfD_s (mg kg^{-1} d^{-1}) and SF (mg kg^{-1} d^{-1}) for seven heavy metals (De Miguel et al, 2007; Ferreira-Baptista and De Miguel, 2005; Cheng et al., 2018; Cao et al., 2015; Jiang et al., 2017).

	Cd	Cr	Cu	Mn	Ni	Pb	Zn
RfD_s for ingestion	1.00E-03	3.00E-03	4.00E-02	4.60E-02	2.00E-02	3.50E-03	3.00E-01
RfD_s for dermal absorption	1.00E-05	6.00E-05	1.20E-02	1.84E-03	5.40E-03	5.25E-04	6.00E-02
SF for ingestion	6.10E+00	5.00E-01	-	-	1.70E+00	8.50E-03	-
SF for dermal absorption	6.10E+00	2.00E+01	-	-	4.25E+01	-	-

The hazard index was then determined by adding the HQs for each variable within study. HI values of below 1.0 indicate that significant additive or harmful interactions were exceedingly improbable and HI values of > 1.0 imply that undesirable, non-carcinogenic health effects were probable (USEPA, 2007; USEPA, 2011).

The carcinogenic risk (CR) was calculated (Eq. 4) by combining the average daily doses (ADD) with the slope factor.

Carcinogenic risk (CR) = Average Daily Dose (ADD) x Slope Factor (SF) (4)

Computed carcinogenic risk values of between $1x10^{-6}$ and $1x10^{-4}$ are considered to be within the acceptable and tolerable risk range for human health (USEPA, 2011).

Statistical Analysis and Spatial Distribution

Descriptive statistics (average, minimum, maximum, standard deviation) were used to determine the distribution of parameters. Data normality was checked and a transformation was performed for some parameters. Then a factor analysis was performed. Principal component analysis (PCA) was performed to identify possible sources of heavy metals. In factor analysis, Barlett and Kaiser–Meyer–Olkin (KMO) tests were used to determine the suitability of the data set, the principal component analysis method was used to determine the factors, and the Varimax technique was used for the rotation process. As a result of factor analysis, groups with eigen values equal to or greater than 1 were accepted as factors. Pearson correlation analysis was performed to determine the relationships between parameters. Statistical analyses were performed with the use of IBM SPSS 17.0 software (SPSS Inc., 2007). Spatial distribution of heavy metal concentrations in mine tailings and soils was performed with the use of ArcGIS 10.1 software (ESRI, 2009).

RESULTS AND DISCUSSION

Some physical and chemical properties of tailing and soil samples taken from Balya and Koru mining areas are provided in Table 4. Balya tailings were 33% loamy-sand, 60% sandy-loam and 7% sandy in texture. Average pH, lime (%), EC (dS m^{-1}) and OM (%) content of mine tailings were found to be 6.1, 2.1, 0.7 and 1, respectively. Average Cd, Cr, Cu, Mn, Ni, Pb and Zn content of tailings were determined to be 35.2, 17.8, 354.7,1734.9, 10, 10089 and 3730 mg kg^{-1}, respectively. Balya soils were all sandy-loam in texture. Soil pH values varied between 6 - 7.3 and EC values varied between 0.3 - 0.9 dS m^{-1}. Average lime (%) and OM (%) content of the soils were determined to be 7.7 and 2.2. Soil heavy metal concentrations were ordered as Pb > Zn > Mn > Cu > Cd > Cr > Ni.

Koru tailings were 33% sandy-clay-loam and 67% sandy-loam in texture. Tailing pH values varied between 4.2 - 6.6, EC values varied between 0.1 - 0.8 dS m^{-1}, lime contents between 0.1 - 1.4% and organic matter contents between 0.4 - 4.7%(Table 1). Average Cd, Cr, Cu, Mn, Ni, Pb, and Zn concentration of tailings were determined to be 9, 8.9, 101.5, 1308, 4.5,1871 and 1375 mg kg^{-1}, respectively. Koru soils were 50% sandy-loam, 25% sand and 25% loamy-sand in texture. Soil average pH, EC (dS m^{-1}), lime (%) and OM (%) contents were determined as 5.7, 0.6, 0.5 and 0.9, respectively. Soil average Cd, Cr, Cu, Mn, Ni, Pb of Zn concentrations were determined to be 4.6, 3.4, 57.5, 1190, 1.6, 431.4 and 909 mg kg^{-1}, respectively.

Table 4. Some physicochemical properties of tailings and soils in Balıkesir-Balya and Çanakkale-Koru Pb-Zn mining areas

	Balya									
	Tailing					Soil				
Parameters	Mean	Std.Deviation	Min.	Max.	Median	Mean	Std.Deviation	Min.	Max.	Median
Clay(%)	9.7	3	4.1	16.7	10.2	14	3	6.3	18.7	14.3
Silt(%)	14.2	6	4.2	27.1	12.8	18.7	4.1	12.3	30.6	18.4
F.S(%)	71.2	8.7	56.2	86.2	70.3	61	5.7	53.3	76.3	60.4
C.S(%)	4.9	2.4	1.5	11.2	4.3	6.3	3	1.1	15.5	6.3
pH	6.1	0.8	3.4	7	6.4	6.9	0.3	6	7.3	7
EC(dS m^{-1})	0.7	0.3	0.2	1.9	0.7	0.6	0.2	0.3	0.9	0.5
Lime(%)	2.1	2.8	0.1	15	1.3	7.7	5.4	1.1	21.1	7
O.M(%)	1.0	0.8	0.03	4.3	0.9	2.2	1.2	0.6	5.1	2
Cd	35.2	29.2	0.2	98.9	30.9	20.4	33.2	5.1	172.7	13
Cr	17.8	23.2	0.2	112.7	8.3	14.4	6.7	2.8	27.1	13.5
Cu	354.7	252.7	18	1207.5	348.4	168.5	259	39.7	1349.5	127.8
Mn	1734.9	640.2	171.8	3182	1878	1271	635	321	2558	1120
Ni	10	10.9	0.1	39.2	7.8	5.6	5.2	0.1	24.3	4.1
Pb	10089	6299	612	27845	9045	6824	4495	1890	26850	6242
Zn	3730	1946	134	6531	4397	2204	1246	1002	6338	1920
	Koru									
	Tailing					Soil				
Parameters	Mean	Std.Deviation	Min.	Max.	Median	Mean	Std.Deviation	Min.	Max.	Median
Clay(%)	15.3	6.3	10.2	26.1	12.3	7.6	3.6	4.1	16.3	6.2
Silt(%)	17.6	2.1	12.3	20.8	18.4	11.7	5.8	4.1	20.2	11.1
F.S(%)	57.4	5.7	43.2	66.5	58.8	75.8	9.1	59.1	90.2	77
C.S(%)	9.7	4.1	1.9	15.2	10.6	4.9	2.8	1	10.5	5.2
pH	5.1	0.7	4.2	6.6	5	5.7	0.6	4.5	6.3	6
EC(dS m^{-1})	0.2	0.1	0.1	0.8	0.2	0.6	0.4	0.1	1.4	0.3
Lime(%)	0.3	0.2	0.1	1.4	0.2	0.5	0.3	0.1	0.9	0.5
O.M(%)	2.1	1.2	0.4	4.7	1.9	0.9	0.7	0.2	1.9	0.6
Cd	9.9	11	1	46.4	5	4.6	2.5	1.2	9.8	4.4
Cr	8.9	6.3	4.5	28.3	6.7	3.4	1.9	1.5	8.5	2.8
Cu	101.5	46.5	28.6	176.6	88	57.5	36	12.9	114.3	49.9
Mn	1308	519	448	2254	1261	1190	685	185	2024	1331
Ni	4.5	1.8	2.8	8.9	3.8	1.6	0.6	0.6	2.7	1.4
Pb	1871	1425	397	4964	1526	431.4	256.8	17.9	773.9	528.9
Zn	1375	824	706	3598	1054	909	589	30	1548	1194

F.S: Fine sand, C.S: Coarse sand, EC: electrical conductivity, O.M: Organic matter

Forghani et al. (2015) took soil samples from agricultural fields and Pb-Zn mining sites of Iran and reported that mining site soils had greater heavy metals levels than the agricultural soils. Sebei et al. (2020) performed heavy metal analyses in soil samples taken from an abandoned mine of Fedj Lahdoum, northern Tunisia and reported heavy metal contamination order as Zn > Pb > Cd. Nassiri et al. (2021) performed heavy metal analyses in soil samples taken from High Moulouya, Zeida abandoned mining area in northeastern Morocco. The hazards index for both adults and children was determined in the order of Mn > Co > Pb > Ni > other heavy metals. The hazards of dermal and inhalation cancer were designated to be negligible.

Comparisons of present heavy metal concentrations with the findings of previous studies conducted on the other Pb-Zn mining regions throughout the world are presented in Table 5. For Balya tailings, Cd concentrations were lower than Slovenia and higher than China, Slovakia, Spain and India; Cr concentrations were higher than China and lower than Slovakia, Spain and India; Cu concentrations were higher than China, Slovakia, Spain and India; Mn contents were higher than Spain and lower than India; Ni contents were higher than China and lower than Spain and India; Pb contents were lower than Slovenia and higher than China, Slovakia, Spain and India; Zn contents were lower than Slovenia and higher than China, Slovakia, Spain and India. For Balya soils, Cd concentrations were higher than the other countries (China, Morocco, Iran, Türkiye and worldwide); Cr concentrations were lower than the others (China, Türkiye and worldwide); Cu contents were higher than the others (China, Morocco, Iran, Türkiye and worldwide); Mn concentrations were higher than Iran and worldwide; Ni contents were lower than the others (China, Türkiye and worldwide); Pb and Zn contents were found to be higher than the other countries (China, Morocco, Iran, Türkiye and worldwide).

Table 5. Average heavy metal concentrations of tailings and soils in Pb-Zn mining areas in the world (in $mgkg^{-1}$)

Material	Location	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Reference
Tailing	Meza,Slovenia	176.3	-	-	-	-	12192	26166	Miler and Gosar, 2012
	Liaoning, China	11	0.7	15.9	-	1.7	649.5	2285	Zhang et al., 2016
	Zlataldka, Slovakia	2.8	69.4	156.6	-	-	1283.1	66.8	Rapant et al., 2006
	Linares, Spain	0.8	50	145	1211	20.6	4077	124	Martinez et al., 2007
	Zawar, India	8.9	41.7	29.5	1798	41	1435	1442	Anju and Banerjee, 2012
	Balya, Türkiye	35.2	17.8	354.7	1342.4	6	10089	3730	This research
	Koru, Türkiye	9.9	3.9	78.2	1308	2.6	1871	1375	This research
Soil	Huan, China	0.2	59.8	30	-	28.4	39.2	112.3	Lu et al., 2015
	Moulouya, Morocco	0.4	-	26.2	-	-	1935.7	47	Azhari et al., 2017
	Irankouh, Iran	2.5	-	38.4	1212.1	-	281.7	1035.2	Jahromi et al., 2020
	Türkiye	3	100	140	-	75	300	300	SPCR, 2005
	Worldwide	0.5	54	19.8	437	22	28.6	64	Kabata-Pendias and Pentias, 2011
	Balya, Türkiye	20.4	14.4	168.5	1864	14.4	6824	2204	This research
	Koru, Türkiye	4.6	10.8	92.5	1190	4.5	431	909	This researh

For Koru tailings, Cd concentrations were found to be lower than Slovenia and China and higher than Slovakia, Spain and India; Cr and Cu contents were higher than China and lower than Slovakia, Spain and India; Mn concentrations were higher than Spain and lower than India; Ni contents were higher than China and lower than Spain and India; Pb concentrations were lower than Slovenia and Spain, but higher than China and India; Zn contents were lower than the other countries (Slovenia, China, Spain, and India. For Koru soils, Cd concentrations were higher than the other countries (China, Morocco, Iran, Türkiye and worldwide); Cr contents were lower than the others (China, Türkiye, worldwide); Cu contents were lower than the other countries (China, Morocco, Iran, Türkiye and worldwide); Mn contents were lower than Iran and higher than the other countries; Ni concentrations were lower than the others (China, Türkiye, worldwide); Pb contents were higher than the others (China, Morocco, Türkiye and worldwide); Zn contents were higher than the others, except for Iran (Table 5).

Correlations between physicochemical properties and heavy metals of mine tailings and soils Balya mining site are demonstrated in Table 6. For Balya tailings, a negative correlation was determined between clay and fine sand (r=0.79), clay and pH (r=0.40), clay and Cd (r=0.32), clay and Cu (r=0.49), clay and Pb (r= 0.50) and clay and Zn (r=0.47); a negative correlation was determined between fine sand and silt (r=0.91), pH and silt (r=0.49), Cd and silt (r=0.43), Cu and silt (r=0.32), Pb and silt (r=0.40), Pb and Zn (r=0.46); a positive correlation was found between silt and EC (r=0.31); a negative correlation was determined between fine sand and coarse sand (r=0.39), fine sand and EC (r=0.32), fine sand and Cd (r=0.43), fine sand and Cu (r=0.41), fine sand and Cr (r=0.42); a positive correlation was determined between fine sand and pH (r=0.44), fine sand and Pb (r=0.46), fine sand and Zn (r=0.50); a positive correlation was determined between coarse sand and Cr (r=0.54); a negative correlation was determined between pH and EC (r=0.49), pH and Ni (r=0.35); a positive correlation was determined between pH and lime (r=0.39), pH and Cd (r=0.61), pH and Cu (r=0.37), pH and Zn (r=0.69); EC and SOM (r=0.37), EC and Cd (r=0.40), EC and Cu (r=0.48), EC and Ni (r=0.29), EC and Pb (r=0.47); a negative significant correlation was determined between EC and Zn (r=0.53); a positive significant correlation was determined between lime and Ni (r=0.61); a significant positive correlation was determined between Cd and Cu

(r=0.66), Cd and Pb (r=0.68), Cd and Zn (r=0.89); a positive significant correlation was determined between Cu and Pb (r=0.86), Cu and Zn (r=0.76); a negative significant correlation was determined between Mn and Zn (r=0.24); a positive significant correlation was determined between Pb and Zn (r=0.77). For Balya soils, a positive correlation was determined between clay and silt (r=0.41), a negative significant correlation was determined between clay and fine sand (r=-0.72), clay and Cu (r=0.51), clay and Pb (r=0.40); a negative significant correlation was determined between silt and fine sand (r=0.74), silt and EC (r=0.25); a negative significant correlation was determined between fine sand and Cd (r=0.52), fine sand and Cu (r=0.56); a negative significant correlation was determined between coarse sand and OM (r=0.41), coarse sand and Cu (r=0.19), pH and Cu (r=0.11); a positive significant correlation was determined between pH and lime (r=0.56), pH and Cr (r=0.67), pH and Zn (r=0.45); a positive significant correlation was determined between pH and Cr (r=0.67); a positive significant correlation was determined between lime and Cr (r=0.75), lime and Ni (r=0.45); a negative significant correlation was determined between lime and Zn (r=0.46); a significant positive correlation was determined between Cd and Cu (r=0.99), Cd and Pb (r=0.93), Cd and Zn (r=0.83); a positive significant correlation was determined between Cr and Ni (r=0.41); a positive significant correlation was determined between Pb and Zn (r=0.64).

Table 6. Pearson correlation coefficient between physicochemical properties of tailings/soils and heavy metals contents for Balya tailings and soils*

Type	Parameters	Clay	Silt	F.S	C.S	pH	EC	$CaCO_3$	OM	Cd	Cu	Cr	Mn	Ni	Pb
Tailing	Silt	0.60													
	F.S	-0.79*	-0.91*												
	C.S	0.15	0.09	-0.39*											
	pH	-0.40*	-0.49*	0.44*	0.12										
	EC	0.25	0.31*	-0.32*	0.08	-0.49*									
	$CaCO_3$	-0.01	-0.12	0.02	0.23	0.39*	-0.12								
	OM	0.29	0.17	-0.16	-0.20	-0.05	-0.37*	-0.26							
	Cd	-0.32*	-0.43*	-0.43*	-0.07	0.61*	-0.40*	-0.04	0.03						
	Cu	-0.49*	-0.32*	-0.41*	-0.08	0.37*	-0.48*	-0.16	-0.01	0.66*					
	Cr	0.28	0.25	-0.42*	0.54*	0.12	0.14	0.21	-0.22	0.04	0.12				
	Mn	0.23	0.20	-0.24	0.06	-0.25	0.01	0.13	0.20	-0.20	-0.02	0.05			
	Ni	-0.16	-0.15	0.12	0.17	-0.35*	-0.29*	0.61*	-0.09	0.14	0.12	0.05	0.12		
	Pb	-0.50*	-0.40*	0.46*	-0.05	0.45	-0.47*	0.02	-0.11	0.68*	0.86*	0.07	-0.03	0.23	
	Zn	-0.47*	-0.46*	0.50*	-0.07	0.69*	-0.53*	-0.09	-0.01	0.89*	0.76*	0.05	-0.24*	0.16	0.77*
Soil	Silt	0.41*													
	F.S	-0.72*	-0.74*												
	C.S	-0.18	-0.36	-0.19											
	pH	0.19	-0.21	-0.03	0.15										
	EC	-0.37	-0.25*	0.24	0.24	-0.14									
	$CaCO_3$	-0.11	-0.24	0.08	0.28	0.56*	0.23								
	OM	0.35	0.33	-0.19	-0.41*	0.11	-0.29	-0.21							
	Cd	-0.51-	-0.23	-0.52*	-0.16	-0.13	0.19	-0.14	-0.03						
	Cu	-0.51*	-0.27	0.56*	-0.19*	-0.11*	0.19	-0.15	0.03	0.99*					
	Cr	-0.28	-0.28	0.21	0.26	0.67*	0.21	0.75*	-0.21	0.03	0.01				
	Mn	-0.11	-0.22	0.04	0.33	-0.21	-0.06	-0.39	-0.18	0.02	0.01	-0.16			
	Ni	-0.33	-0.42	0.35	0.23	0.09	0.31	0.45*	-0.36	0.20	0.18	0.41*	0.13		
	Pb	-0.40*	-0.19	0.40*	-0.11	0.16	0.11	0.12	0.09	0.93*	0.93*	0.24	-0.09	0.21	
	Zn	-0.37	-0.13	0.37	-0.15	-0.45*	0.35	-0.46*	-0.02	0.83*	0.83*	-0.38	0.07	0.06	0.64*

F.S: fine sand, C.S: coarse sand, EC: electrical conductivity, OM: organic matter, *p < 0.05

Correlations between physicochemical properties and heavy metals of tailings and soils of Koru mining site are demonstrated in Table 7. For Koru tailings, a negative correlation was determined between clay and fine sand (r=0.71), clay and coarse sand (r=0.53); a positive significant correlation was determined between clay and pH (r=0.55), clay and EC (r=0.59), clay and lime (r=0.51); a negative significant correlation was determined between fine sand and coarse sand (r=0.13); a significant negative correlation was determined between coarse sand and pH (r=0.66), coarse sand and EC (r=0.63), coarse sand and lime (r=0.73), coarse sand and Zn (r=0.60); a positive significant correlation was determined between coarse sand and OM (r=0.60); a positive significant correlation was determined between pH and lime (r=0.72), pH and Cr (r=0.54), pH and Zn (r=0.66); a positive significant correlation was determined between EC and lime (r=0.52); a negative significant correlation was determined between EC and OM (r=0.57); a

negative significant correlation was determined between lime and OM (r=0.49); a positive significant correlation was determined between Cd and Pb (r=0.65); a positive significant correlation was determined between Cu and Zn (r=0.49); a positive significant correlation was determined between Mn and Zn (r=0.54). For Koru soils, a negative correlation was determined between clay and fine sand (r=0.84), clay and pH (r=0.77), clay and lime (r=0.60); a positive correlation was determined between clay and OM (r=0.87), clay and Cd (r=0.73); a negative correlation was determined between silt and fine sand (r=0.82); a positive correlation was determined between silt and OM (r=0.58); a negative correlation was determined between fine sand and OM (r=0.87); a negative correlation was determined between fine sand and Cd (r=0.60); a positive correlation was determined between fine sand and pH (r=0.79); a positive correlation was determined between pH and lime (r=0.87); a negative correlation was determined between pH and OM (r=0.83), pH and Cd (r=0.76); a positive correlation was determined between EC and lime (r=0.19); a negative correlation was determined between EC and Cu (r=0.72), EC and Zn (r=0.64); a negative correlation was determined between lime and OM (r=0.70), lime and Cd (r=0.69); a positive correlation was determined between OM and Cd (r=0.78); a positive correlation was determined between Cu and Ni (r=0.71); a positive correlation was determined between Cr and Mn (r=0.64), Cr and Pb (r=0.74), Cr and Zn (r=0.76); a positive correlation was determined between Mn and Zn (r=0.89); a positive correlation was determined between Pb and Zn (r=0.92).

Table 7. Pearson correlation coefficient between physicochemical properties of tailings/soils and heavy metals contents for Koru tailings and soils*

Type	Parameters	Clay	Silt	F.S	C.S	pH	EC	Lime	OM	Cd	Cu	Cr	Mn	Ni	Pb
Tailing	Silt	-0.03													
	F.S	-0.71*	-0.32												
	C.S	-0.53*	-0.02	-0.13*											
	pH	0.55*	0.02	-0.14	-0.66*										
	EC	0.59*	0.33	-0.31	-0.63*	0.34									
	Lime	0.51*	0.02	-0.04	-0.73*	0.72*	0.52*								
	OM	-0.44	0.15	-0.01	0.60*	-0.32	-0.57*	-0.49*							
	Cd	0.02	-0.08	0.05	-0.04	-0.08	0.14	0.12	-0.40						
	Cu	0.06	0.25	-0.16	0.01	0.01	0.02	0.26	0.28	0.10					
	Cr	0.11	-0.14	0.05	-0.16	0.54*	-0.09	0.65*	-0.14	0.04	0.03				
	Mn	0.36	0.39	-0.37	-0.24	0.42	0.22	0.32	-0.27	0.02	0.30	0.12			
	Ni	-0.05	0.09	-0.36	0.53*	-0.15	-0.28	-0.21	0.42	-0.12	0.35	-0.15	0.01		
	Pb	-0.16	-0.33	0.34	-0.06	0.06	-0.15	0.38	-0.19	0.65*	0.44	0.17	-0.11	-0.06	
	Zn	0.16	0.06	0.24	-0.60*	0.66*	0.18	0.61*	-0.52*	0.40	0.49*	-0.08	0.54*	-0.34	0.38
Soil	Silt	0.48													
	F.S	-0.84*	-0.82*												
	C.S	0.42	-0.02	-0.46											
	pH	-0.77*	*0.66*	0.79*	-0.21										
	EC	-0.23	-0.25	0.34	-0.28	0.15									
	Lime	-0.60*	-0.43	0.53	-0.04	0.87*	0.19*								
	OM	0.87*	0.58*	-0.87*	0.48	-0.83*	-0.34	-0.70*							
	Cd	0.73*	0.28	-0.60*	0.44	-0.76*	-0.52	-0.69*	0.78*						
	Cu	-0.08	0.06	-0.09	0.27	-0.11	-0.72*	-0.22	0.25	0.35					
	Cr	0.15	0.57	-0.38	-0.14	0.04	-0.21	0.12	0.10	-0.33	-0.07				
	Mn	0.12	0.05	-0.07	-0.05	-0.18	-0.77	-0.30	0.26	0.56	-0.12	0.64*			
	Ni	0.42	0.35	-0.42	0.10	-0.19	0.06	-0.11	0.20	-0.13	0.71*	-0.30	-0.24		
	Pb	0.21	0.23	-0.28	0.16	-0.44	-0.73	-0.55	0.48	0.71	-0.22	0.74*	0.88*	-0.29	
	Zn	-0.08	0.13	-0.01	-0.01	-0.18	-0.64*	-0.36	0.24	0.42	-0.15	0.76*	0.89*	-0.38	0.92*

F.S: fine sand, C.S: coarse sand, EC: electrical conductivity, OM: organic matter, *p < 0.05

For Balya mining site, three factors explained 78.61% of total variation in tailings and 74.79% of the total variance in soils (Table 8). It is known that Cd mainly occurs in association with sphalerite (ZnS) (Tran et al., 2022). Pb and Cu originate from galena (PbS) and chalcopyrite ($CuFeS_2$) minerals. These elements are also known to be often originated from Pb–Zn mining activities (Cao et al., 2022; Jahromi et al., 2020; Nassiri et al., 2021). For Balya tailings, the first factor included Cd, Pb and Cu (41.85% of total variance), the second factor included Mn (20.70% of total variance) and the third factor included Zn (16.04% of total variance). For Balya soils, Factor 1 (32.51% of total variance) included Cr; Factor 2 (24.70% of total variance) included Ni; Factor 3 (17.58% of total variance) included Mn.

Table 8. Principal components of mine tailings and soil samples in Balya

	Mine tailing			Soil		
	Factor 1	**Factor 2**	**Factor 3**	**Factor 1**	**Factor 2**	**Factor 3**
Mn	-0.303	**0.738**	0.290	-0.038	-0.088	**0.925**
Cr	0.235	0.694	-0.460	**0.795**	0.353	-0.056
Cd	**0.895**	-0.154	0.109	0.178	-0.806	0.308
Pb	**0.909**	0.225	-0.080	0.493	-0.453	-0.577
Zn	0.032	0.017	**0.935**	-0.764	0.003	0.032
Ni	0.403	0.657	-0.056	0.264	**0.807**	0.325
Cu	**0.892**	0.177	-0.116	-0.775	0.195	0.063
Eigenvalues	2.930	1.450	1.123	2.275	1.729	1.231
% of variance	41.858	20.708	16.039	32.506	24.701	17.580
% cumulative variance	41.858	62.566	78.606	32.506	57.208	74.788
Kaiser- Meyer- Olkin measure of sampling adequacy			0.638		0.488	
Bartlett's test of sphericity			0.000		0.003	

*Bold values are factor loadings of the principal components

For Koru mining site, three factors explained 71.78% of total variance in tailings and two factors explained 64.85% of total variance in soils (Table 9). For Koru tailings, Factor 1 (33.33% of total variance) included Mn and Cr; Factor 2 (20.17 % of total variance) included Cd, Pb, and Zn; Factor 3 (18.28% of total variance) included Ni. For Koru soils, Factor 1 (42.22% of total variance) included Mn and Cu; Factor 2 (22.63% of total variance) included Cr and Ni (Table 4). Tran et al. (2022) reported that heavy metals originate from combination of anthropogenic and geogenic sources in soils near Pb-Zn mining areas.

Table 9. Principal components of mine tailings and soil samples in Koru

	Mine tailing			Soil	
	Factor 1	**Factor 2**	**Factor 3**	**Factor 1**	**Factor 2**
Mn	**0.872**	0.006	0.080	**0.921**	0.034
Cr	-0.777	-0.022	0.202	-0.085	**0.872**
Cd	0.210	**0.809**	-0.197	0.634	-0.182
Pb	-0.272	**0.769**	0.045	0.122	-0.769
Zn	0.602	**0.704**	0.023	-0.548	0.264
Ni	-0.057	-0.221	**0.809**	-0.224	**0.886**
Cu	0.030	-0.091	-0.794	**0.814**	-0.111
Eigenvalues	2.333	1.412	1.280	2.956	1.584
% of variance	33.325	20.171	18.281	42.223	22.626
% cumulative variance	33.325	53.497	71.778	42.223	64.849
Kaiser- Meyer- Olkin measure of sampling adequacy			0.407		0.473
Bartlett's test of sphericity			0.094		0.078

*Bold values are factor loadings of the principal components

Local distribution maps of heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) of Balya mining site are presented in Figure 3. For Balya tailings, the lowest Cd, Cr, Cu, Pb and Zn values were found in Region 2 and the lowest Mn and Ni levels were seen in Region 3. The highest Cd and Cu values were seen in Region 5 (S8- Region 5 with 172.70 mg kg^{-1} Cd and 1349.5 mg kg^{-1} Cu). The highest Pb, Zn and Mn concentrations were seen in Region 3 (27845 mg kg^{-1} Pb, 6530.5 mg kg^{-1} Zn and 3182 mg kg^{-1} Mn). In general, average of heavy metal concentrations of tailings were higher than the soils. Although heavy metal concentrations were low in Region 1, it is noteworthy that the heavy metal concentrations in Region 3 were high.

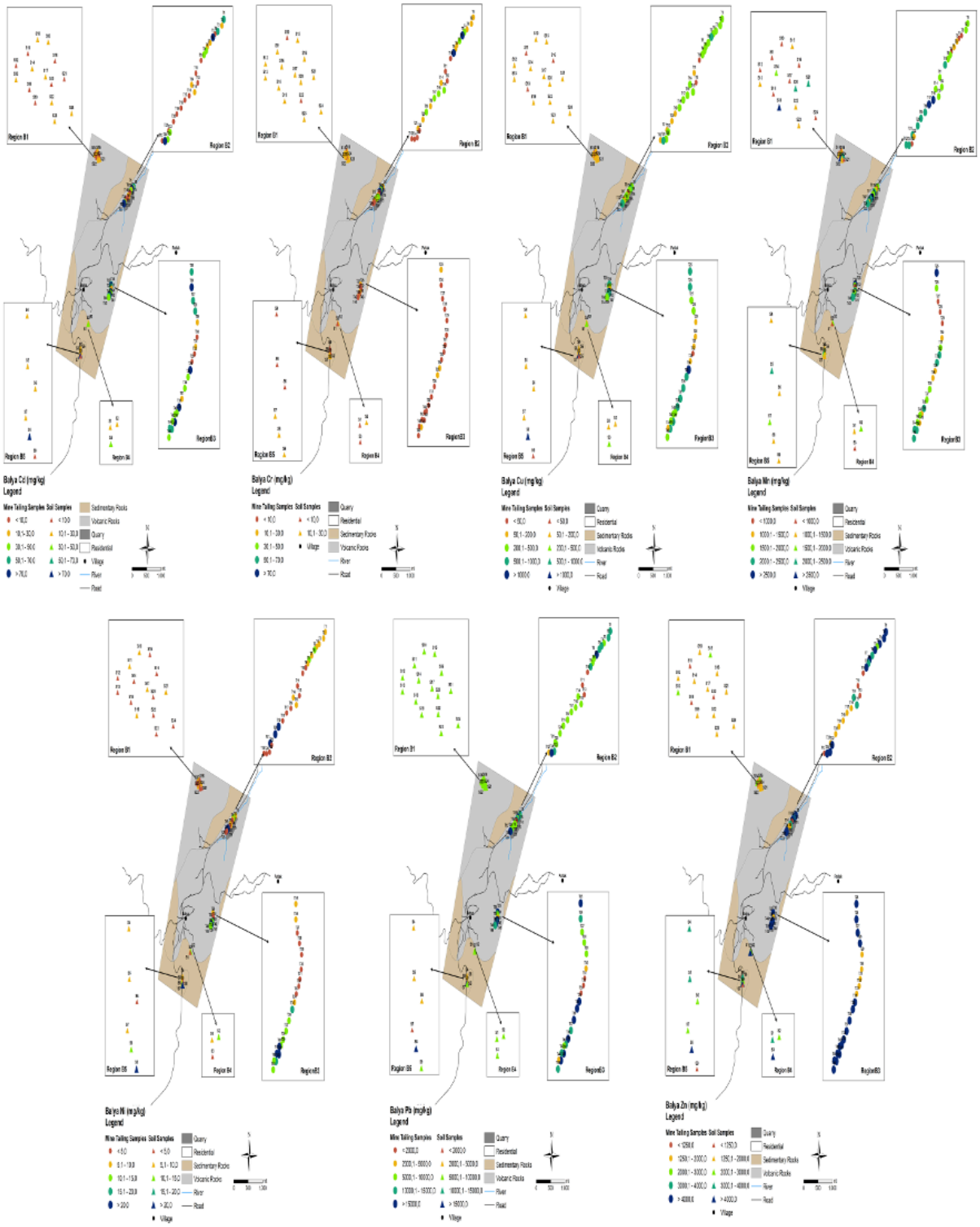

Figure 3. Spatial distribution of heavy metals in tailings and soils in the Balya mining area

Local distribution maps of heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) of Koru mining site are presented in Figure 4. The lowest Cr and Ni concentrations were found in soil samples (S4) and the highest concentrations were found in tailing samples T2 and T7. The lowest Mn and Zn concentrations were found in soil sample S8 (185.28 mg kg^{-1} and 30.09 mg kg^{-1}) and the highest Mn and Zn concentrations were found in T2 tailing sample (2254 mg kg^{-1} and 3598 mg kg^{-1}). The lowest Cu and Pb concentrations were found in soil sample S7 (12.89 mg kg^{-1} and 17.88 mg kg^{-1}) and the highest Cu and Pb concentrations were found in T18 and T2 tailing samples (176.62 mg kg^{-1} and 4963.5 mg kg^{-1}). In general, average Cd, Cr, Cu, Mn, Ni, Pb and Zn concentrations of tailing samples were greater than the soil samples.

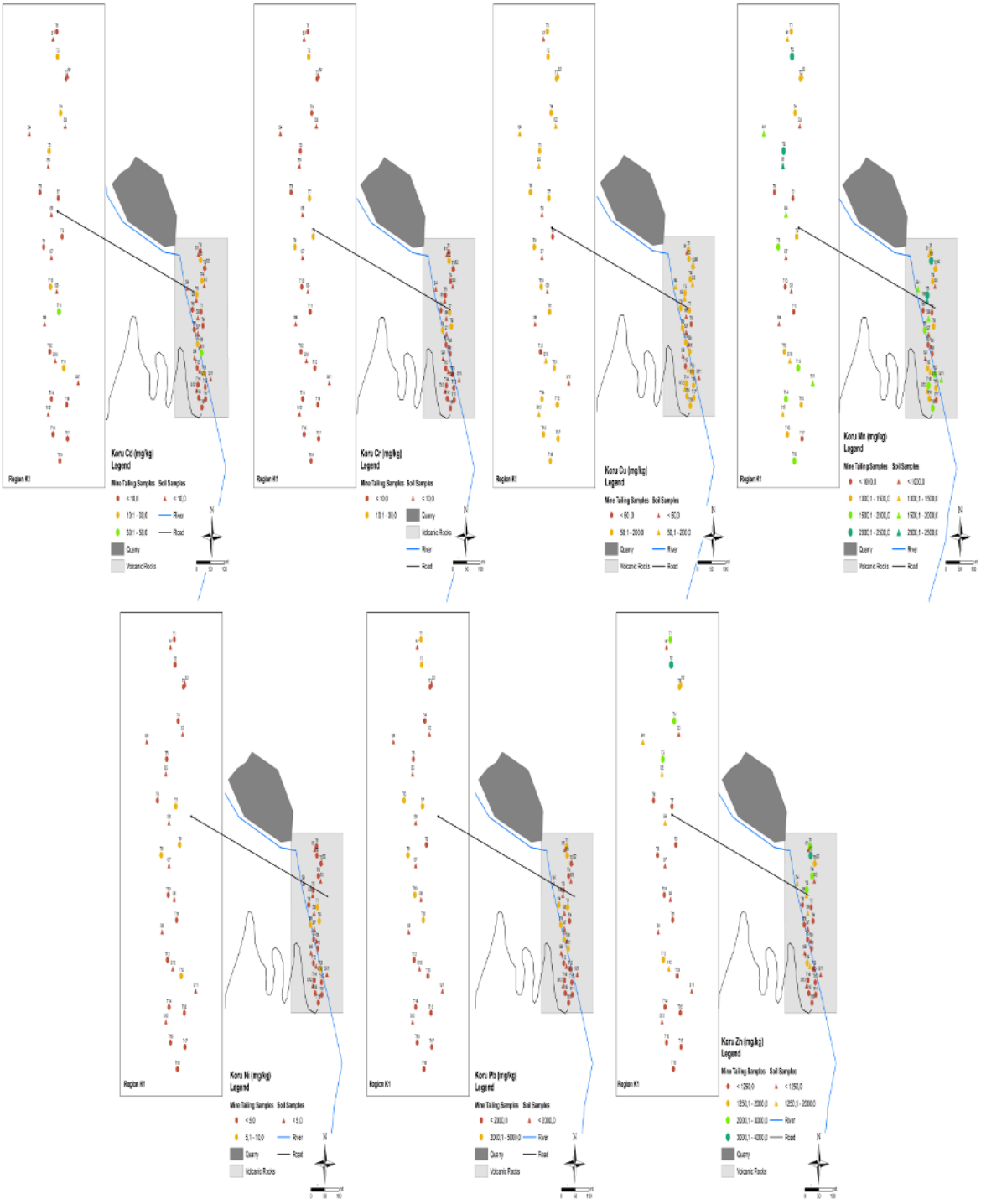

Figure 4. Spatial distribution of heavy metals in tailings and soils in the Koru mining area

The ADD values for adults are presented in Table 10. For Balya mining site, ADD values varied between 1.02E-03 and 1.65E-10 in tailing samples and between 9.32E-01 and 2.39E-10 in soil samples. For Koru mining site, ADD values for adults varied between 3.58E-04 and 7.46E-11 in tailing samples and between 1.18E-04 and 2.65E-11 in soil samples.

Table 10. ADD values for adults in mine tailing and soils in Balya and Koru mining areas

Sample Type	Heavy metals	Balya		Koru	
		ADD_{ing}	ADD_{derm}	ADD_{ing}	ADD_{derm}
Mine tailing	Cd	9.64E-06	5.82E-10	2.73E-06	1.64E-10
	Cr	4.88E-06	2.94E-10	2.45E-06	1.48E-10
	Cu	9.72E-05	5.86E-09	2.78E-05	1.67E-09
	Mn	4.75E-04	2.86E-08	3.58E-04	2.16E-08
	Ni	2.74E-06	1.65E-10	1.24E-06	7.46E-11
	Pb	2.76E-03	1.67E-07	5.13E-04	3.09E-08
	Zn	1.02E-03	6.16E-08	3.77E-04	2.27E-08
Soil	Cd	5.60E-06	3.38E-10	1.27E-06	7.69E-11
	Cr	3.96E-06	2.39E-10	9.20E-07	5.55E-11
	Cu	4.62E-05	2.78E-09	1.58E-05	9.50E-10
	Mn	3.48E-04	2.10E-08	3.26E-04	1.96E-08
	Ni	1.54E-06	9.32E-01	4.39E-07	2.65E-11
	Pb	1.87E-03	1.13E-07	1.18E-04	7.13E-09
	Zn	6.04E-04	3.64E-08	2.49E-04	1.50E-08

The ADD values for children are presented in Table 11. For Balya mining site, ADD values for children varied between 3.83E-02 and 9.15E-10 in tailing samples and between 2.59E-02 and 5.77E-10 in soil samples. For Koru mining site, ADD values for children varied between 7.11E-03 and 9.15E-10 in tailing samples and between 4.52E-03 and 1.64E-10 in soil samples.

Table 11. ADD values for children in mine tailing and soils in Balya and Koru mining areas

Sample Type	Heavy metals	Balya		Koru	
		ADD_{ing}	ADD_{derm}	ADD_{ing}	ADD_{derm}
Mine tailing	Cd	1.34E-04	3.60E-09	3.78E-05	1.02E-09
	Cr	6.77E-05	1.82E-09	3.40E-05	9.15E-10
	Cu	1.35E-03	3.62E-08	3.86E-04	1.03E-08
	Mn	6.59E-03	1.77E-07	4.97E-03	1.33E-07
	Ni	3.80E-05	1.02E-09	1.71E-05	4.61E-10
	Pb	3.83E-02	1.03E-06	7.11E-03	1.91E-07
	Zn	1.42E-02	3.82E-07	5.22E-03	1.41E-07
Soil	Cd	7.76E-05	2.09E-09	1.77E-05	4.76E-10
	Cr	5.49E-05	1.48E-09	1.28E-05	3.44E-10
	Cu	6.40E-04	1.72E-08	2.19E-04	5.88E-09
	Mn	4.83E-03	1.29E-07	4.52E-03	1.21E-07
	Ni	2.14E-05	5.77E-10	6.08E-06	1.64E-10
	Pb	2.59E-02	6.98E-07	1.64E-03	4.41E-08
	Zn	8.37E-03	2.25E-07	3.46E-03	9.30E-08

Health risk assessments for carcinogenic and non-carcinogenic elements of Balya mining site are presented in Table 12. For non-carcinogenic effects on adults, heavy metals were ordered as Pb > Ni > Mn > Cd > Zn > Cu > Cr in tailing samples and Pb > Cd > Mn > Zn > Cr > Cu > Ni in soil samples. For children, heavy metals were ordered as Cd > Pb > Mn > Zn > Cu > Cr > Ni in tailing samples and Cd > Pb > Mn > Zn > Cr > Cu > Ni in soil samples. For children and adults, carcinogenic effects of heavy metals were ordered as Cd > Pb > Ni > Cr. The values of the cancer threat of Cd and Pb were found to be above 1×10^{-4}.

Table 12. Health risk assessment of heavy metal based on total heavy metals in mine tailing and soil for adults and children in Balya

Sample type	Heavy metals	Adults				Children			
		HQ_{ingest}	HQ_{dermal}	HI	Risk	HQ_{ingest}	HQ_{dermal}	HI	Risk
Mine tailing	Cd-non cancer	9.64E-03	5.82E-05	9.70E-03		1.34E-01	1.34E+04	1.34E+04	
	Cd-cancer	5.88E-05	3.54E-09		5.88E-05	8.16E-04	2.19E-08		8.16E-04
	Cr-non cancer	1.63E-03	4.91E-07	1.63E-03		2.26E-02	3.04E-06	2.26E-02	
	Cr- cancer	2.44E-06	5.88E-09		2.44E-06	3.38E-05	3.64E-08		3.38E-05
	Cu	2.43E-03	4.88E-06	2.43E-03		3.37E-02	3.02E-05	3.37E-02	
	Mn	1.03E-02	1.56E-05	1.03E-02		1.43E-01	9.64E-05	1.43E-01	
	Ni-non cancer	1.37E-03	3.06E-08	1.37E-03		1.90E-02	1.90E-07	1.90E-02	
	Ni- cancer	4.66E-06	7.03E-09		4.66E-06	6.46E-05	4.35E-08		6.46E-05
	Pb-non cancer	7.90E-01	3.18E-04	7.90E-01		1.10E+01	1.97E-03	1.10E+01	
	Pb- cancer	2.35E-05			2.35E-05	3.26E-04			3.26E-04
	Zn	3.41E-03	1.03E-06	3.41E-03		4.72E-02	6.36E-06	4.72E-02	
Soil	Cd-non cancer	5.60E-03	3.38E-05	5.63E-02		7.76E-02	7.76E+03	7.76E+03	
	Cd-cancer	3.41E-05	2.06E-09		3.41E-05	4.73E-04	1.27E-08		4.73 E-04
	Cr-non cancer	1.32E-03	3.98E-07	1.32E-03		1.83E-02	2.46E-06	1.83E-02	
	Cr- cancer	1.97E-06	4.78E-09		1.98E-06	2.31E-04	6.20E-09		2.74E-05
	Cu	1.15E-03	2.31E-06	1.16E-03		1.60E-02	1.43E-05	1.60E-02	
	Mn	7.57E-03	1.14E-05	7.58E-03		1.05E-01	7.06E-05	1.05E-01	
	Ni-non cancer	7.72E-04	1.73E-08	7.73E-04		1.07E-02	1.07E-07	1.07E-02	
	Ni- cancer	2.63E-06	3.96E-09		2.63E-06	3.64E-05	2.45E-08		3.64E-05
	Pb-non cancer	5.34E-01	2.15E-04	5.34E-01		7.41E+00	1.33E-03	7.41E+00	
	Pb-cancer	1.59E-05			1.59E-05	2.20E-04			2.20E-04
	Zn	2.01E-03	6.07E-07	2.01E-03		2.79E-02	3.76E-06	2.79E-02	

Health risk assessments for carcinogenic and non-carcinogenic elements of Koru mining site are presented in Table 13. For non-carcinogenic effects on adults, heavy metals were ordered as Pb > Mn > Cd > Zn > Cr > Cu > Ni in tailing samples and Pb > Mn > Cd > Zn > Cu > Cr > Ni in soil samples. For children, heavy metals were ordered as Cd > Pb > Mn > Zn > Cr > Cu > Ni in tailing samples and Cd > Pb > Mn > Zn > Cu > Cr > Ni in soil samples. For children and adults, carcinogenic effects of heavy metals were ordered as Cd > Pb > Ni > Cr. . Cd has a carcinogenic effect for children in both tailings and soils in Koru. Wang et al. (2017) conducted a study in Huedehong lead-zinc mining area (Yunnan-Southwest China) and reported heavy metal contents in the order of Zn > Pb > Cr > Cu > Cd. Cd had the highest non-carcinogenic effect in the study area and Cr had the least cancer risk. Baghaie and Aghili (2019) determined that Cd and Pb concentrations of soil samples taken from Shahin (Iran) mine were higher than the background. Non-cancerogenic hazard quotient values of Pb and Cd were less than 1 for the soils examined. Kan et al. (2021) conducted health risk assessment of heavy metals in Pb-Zn mining areas located in the southern and eastern regions of China and indicated that heavy metals in mine tailings were mostly taken by oral ingestion and children were more sensitive to adverse effects. Nassiri et al. (2021) performed heavy metal analyses in the soil samples taken from the Zeida abandoned mining area in northeastern Morocco, High Moulouya. The hazard index for children and adults was determined in the order of Mn > Co > Pb > 1 > other heavy metals. Inhalation and dermal carcinogenic risks were found to be negligible. Nikolaidis et al. (2013) conducted heavy metal analyses in the samples taken from a lead-zinc mining area in Kirki region (NE Greece) and indicated that arsenic caused health risks.

Table 13. Health risk assessment of heavy metal based on total heavy metals in mine tailing and soil for adults and children in Koru

Sample type	Heavy metals	Adults				Children			
		HQ_{ingest}	HQ_{dermal}	HI	Risk	HQ_{ingest}	HQ_{dermal}	HI	Risk
Mine tailing	Cd-non cancer	2.73E-03	1.64E-05	2.74E-03		3.78E-02	3.78E+03	3.78E+03	
	Cd-cancer	1.66E-05	1.001E-09		1.66E-05	2.31E-04	6.20E-09		2.31E-04
	Cr-non cancer	8.17E-04	2.46E-07	8.17E-04		1.13E-02	1.52E-06	1.13E-02	
	Cr- cancer	1.22E-06	2.95E-09		1.22E-06	1.70E-05	1.82E-08		1.70E-05
	Cu	6.95E-04	1.39E-06	6.97E-04		9.64E-03	8.65E-06	9.65E-03	
	Mn	7.79E-03	1.17E-05	7.80E-03		1.08E-01	7.27E-05	1.08E-01	
	Ni-non cancer	6.18E-04	1.38E-08	6.18E-04		8.57E-03	8.55E-08	8.57E-03	
	Ni- cancer	2.10E-06	3.16E-09		2.10E-06	2.91E-05	1.96E-08		2.91E-05
	Pb-non cancer	1.46E-01	5.89E-05	1.47E-01		2.03E+00	3.65E-04	2.03E+00	
	Pb- cancer	4.36E-06			4.36E-06	6.04E-05			6.04E-05
	Zn	1.26E-03	3.79E-07	1.26E-03		1.74E-02	2.34E-06	1.74E-02	
Soil	Cd-non cancer	1.27E-03	7.69E-06	1.28E-03		1.77E-02	1.77E+03	1.77E+03	
	Cd-cancer	7.78E-06	4.69E-10		7.78E-06	1.08E-04	2.90E-09		1.08E-04
	Cr-non cancer	3.07E-04	9.25E-08	3.07E-04		4.25E-03	5.73E-07	4.25E-03	
	Cr- cancer	4.60E-07	1.11E-09		4.60E-07	6.38E-06	6.87E-09		6.38E-06
	Cu	3.94E-04	7.92E-07	3.95E-04		5.46E-03	4.90E-06	5.47E-03	
	Mn	7.09E-03	1.07E-05	7.10E-03		9.83E-02	6.62E-05	9.84E-02	
	Ni-non cancer	2.19E-04	4.90E-09	2.19E-04		3.04E-03	3.03E-08	3.04E-03	
	Ni- cancer	7.45 E-07	1.12E-09		7.45 E-07	1.03E-05	6.95E-09		1.03E-05
	Pb-non cancer	3.38E-02	1.36E-05	3.38E-02		4.68E-01	8.41E-05	4.68E-01	
	Pb-cancer	1.00E-06			1.00E-06	1.39E-05			1.39E-05
	Zn	8.31E-04	2.51E-07	8.31E-04		1.15E-02	1.55E-06	1.15E-02	

CONCLUSION

In this study, heavy metal concentrations of tailing and soil samples taken from Balya and Koru mining sites were determined and effects of heavy metals on human health were assessed. In both mining sites, heavy metal concentrations were generally higher in tailing samples than in soil samples. The shortcoming of this research is that no sampling was done from the water and sediment in the study area and heavy metal contents were not determined. Mine tailings and soils in the study areas were heavily polluted by Pb and Zn. The non-carcinogenic risk was determined to be at the highest level for Pb and the carcinogenic risk was determined to be at the highest level for Cd and Pb. An environmental management plan should be applied and a soil remediation strategy should be developed to reduce the effects of mining activities on human health.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics Committee Approval

Ethics committee approval is not required.

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Data availability

Not applicable.

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Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Ondokuz Mayıs University drinking water treatment plant carbon footprint: emission sources and strategies for sustainability

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Abstract

In this study, it was aimed to calculate the carbon footprint of the drinking water treatment facility within Samsun Ondokuz Mayıs University and to determine the greenhouse gas emission sources within the facility. The total daily CO_2 emission of the drinking water treatment plant is calculated as 85.05 kg CO_2e/d. The analyses show that off-site CO_2 emissions are higher than on-site CO_2 emission values. It has been determined that the primary source of off-site emissions arises from the electrical energy consumption used in the units, constituting approximately 86.4% of the total CO_2 emissions. The second important contribution parameter arises from the reaction of coagulants in mechanical mixing processes. This study emphasizes the importance of taking measures that support greener and sustainable production to reduce the current greenhouse gas emissions of the facility. In addition, calculating the carbon emissions of the drinking water treatment plant is important in informing the relevant institutions in the European Union's efforts to achieve the goal of zeroing carbon emissions by 2050.

Keywords: Carbon footprint, Greenhouse gases, Drinking water treatment plant, Sustainable water management, Climate change

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INTRODUCTION

Climate change is a worldwide problem today. One of the main causes of climate change is greenhouse gases, and their amounts have continued to increase since the industrial revolution (Clabeaux Et Al., 2020; Coşkun & Doğan, 2021). Although it is stated that the activities that contribute the most to greenhouse gas emissions are in private sectors (iron or steel production and cement clinker production, etc.), it is known that public facilities such as incineration plants and water treatment plants release significant amounts of greenhouse gases (Bani Shahabadi et al., 2009). Recently, it has been known that water treatment plants consume a huge amount of electricity and chemicals, causing significant amounts of CO_2 emissions (Rothausen & Conway, 2011). Although CH_4 and N_2O emissions from drinking water treatment plants are much less than those from wastewater treatment plants, annual greenhouse gas emissions cannot be ignored (Kyung et al., 2013). In the near future, treatment plants will likely be strictly regulated and controlled by protocols. Therefore, CO_2 emissions from water treatment plants must be reduced quickly and managed appropriately.

Carbon footprint calculation is required to reveal the hidden environmental impact of the drinking water treatment plant and take a more environmentally friendly approach to water consumption. Carbon footprint is used to define the amount of greenhouse gas emissions in terms of CO_2 equivalent caused directly and indirectly by an individual, product, industry, city, and region over a certain period (Karakaş, 2021; Yüksel, 2017). Although direct comparison of findings is

somewhat complicated due to different system boundaries, water sources, treatment steps, functional units, and methods in drinking water treatment plants, overall, the use of electricity and chemicals are the main contributors to the carbon footprint in drinking water production (Bonton et al., 2012; Hofs et al., 2022). Nowadays, due to the increasing sensitivity to the environment, the calculation of carbon emissions as carbon footprints and the carbon zeroing policies of industries have begun to be used frequently. It is aimed to reduce negative impacts and emissions by making carbon footprint calculations in many sectors and areas. Carbon footprint calculations have gained importance in our country due to the green agreement process and circular economy studies. In this context, the "Regulation on the Monitoring of Greenhouse Gas Emissions" published by the Ministry of Environment, Urbanization and Climate Change in the Official Gazette No. 29003 in May 2014 came into force (Ministry of Environment, 2014). According to this regulation, industries and businesses that need to monitor greenhouse gases are required to submit their "Greenhouse gas monitoring and emission" plans to the Ministry of Environment, Urbanization and Climate Change. However, treatment facilities are excluded from the regulation and notification. However, treatment facilities have greenhouse gas emissions and need to develop policies to reduce them.

The carbon footprint of drinking water involves a complex web of factors, including water collection, treatment, distribution, and waste management. Each of these stages can directly or indirectly contribute to greenhouse gas emissions. Studies on the carbon emissions of drinking water treatment plants are few, other than life cycle assessment, and there is a serious gap in the literature (Hofs et al., 2022; Vince et al., 2008). These studies compare the carbon footprints of drinking water facilities using conventional and advanced treatment (Bonton et al., 2012; Kyung et al., 2013). Recently, it has become important to reduce CO_2 emissions by providing basic information about important carbon emission sources and their emission amounts with a life cycle assessment approach or mathematical models (Bani Shahabadi et al., 2010; Larsen, 2015; Yan et al., 2014). The correct mathematical model can be selected by deciding on the facility optimization and treatment units. In this context, first of all, the treatment units of the facility must be determined in detail, and then the emission sources inside and outside the facility must be determined. The uniqueness of this study is that although the carbon emissions of wastewater treatment facilities (Güller & Balcı, 2018; Karakaş, 2021) have been calculated in our country, the carbon emissions of drinking water treatment facilities have not been calculated. This study is the first study conducted in our country and aims to set an example for other studies.

In this study, it was aimed to calculate the carbon footprint of the drinking water treatment facility located at Ondokuz Mayıs University (OMU), which is taking firm steps towards becoming a green university. For this purpose, a mathematical model developed by the South Korean government is planned to be used to determine on-site and off-site CO_2 emissions of drinking water (Korea Water Resources Corporation, 2017). It is aimed to provide basic information about CO_2 emission sources by detecting on-site and off-site emissions occurring in the drinking water treatment plant and to provide suggestions for reducing CO_2 emissions efficiently.

MATERIALS AND METHODS

Study area

OMU Kurupelit Campus has an area of 8 thousand 800 decares and has 58 thousand students, 2 thousand 348 academic staff, and 3937 employees, and clean water is delivered to the faculties from the drinking water treatment facility within the university. OMU drinking water treatment plant was established approximately thirty-five years ago to provide clean water to the campus. It has undergone many renovations and revisions over time. Raw water to the facility comes from the Karakavuk pond, located 8.5 km away, and the facility purifies the flow rate of 122 m^3/h daily. Physicochemical measurement results of raw water values coming to the facility are given in Table 1.

Calculation and determination of system limits

In this study, the mathematical model developed by the South Korean government was used to calculate the carbon emissions of the classical drinking water treatment plant (Korea Water Resources Corporation, 2017). It was aimed to estimate the on-site and off-site CO_2 emissions generated in the drinking water treatment plant. In-site CO_2 emissions are defined as emissions resulting from mechanical mixing processes (rapid and slow mixing) and chemical reactions such as the addition of alum ($Al_2(SO_4)_3.18H_2O$). Off-site CO_2 emissions include electricity consumption for treatment units. CH_4 and N_2O emissions were not considered in the model because they are rarely produced in drinking water treatment plants. Additionally, since the amount of sludge generated in the facility is low, no energy is consumed for sludge disposal, and it is discharged to the receiving environment. The production and transportation of chemicals coming to the facility are not included in the system boundaries. The boundaries and emission paths of the OMU drinking water treatment plant system are shown in Figure 1. The operating conditions and parameters of the drinking water treatment plant were used to inform the model that calculates CO_2 emissions. The operating conditions and parameters of the drinking water treatment plant used for input data for the model are given in Table 2.

Table 1. Physicochemical measurement results of raw water coming to the facility.

Parameters	Raw water
pH	8.00
Turbidity (NTU)	64.3
Conductivity (µs/cm)	136.6
Hardness (mg/L)	21
Total suspended solid (mg/L)	80.6
Salinity (‰)	0.1
Temperature (°C)	14
Nitrate nitrogen (NH_3-N)	10.91
Chlorine (Cl^-)	0
Sulfate ($SO4^{-2}$) (mg/L)	100
Manganese (Mn^{+2}) (µg/L)	55
Iron (Fe^{+2}) (µg/L)	680
Aluminum (Al^{+3}) (mg/L)	15
Dissolved oxygen (mg/L)	10.46
Total organic carbon (TOC) (mg/L)	2.91

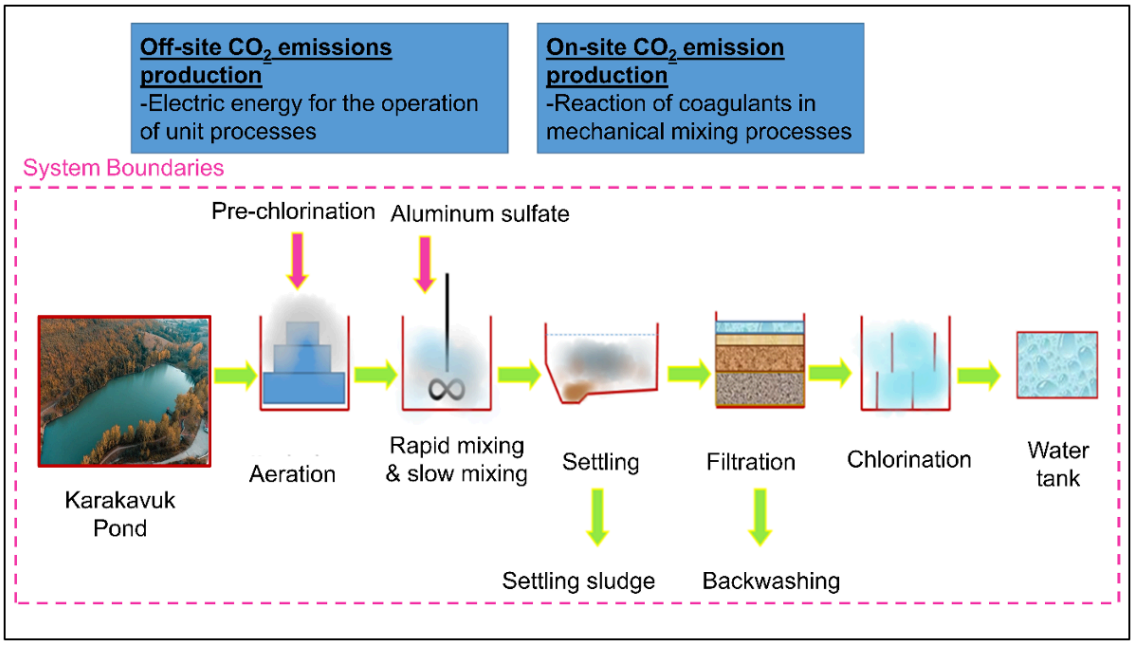

Figure 1. System boundaries and emission sources of OMU drinking water treatment plant.

Table 2. Physicochemical OMU drinking water treatment plant operating conditions and parameters.

Operating Conditions	Symbol	Parameter
Flow	Q_{flow}	2928 m^3/d
input blur	$Turb._{inlet}$	64.3 NTU
Output blur	$Turb._{outlet}$	0.49 NTU
Heat	T	14 °C
water density	P_{water}	998.2 kg/m^3
Operating Parameters		
Chemicals		
Added aluminum sulphate flow rate	Q_{chem}	0.5 m^3/d
Added sodium hypochlorite flow rate	Q_{NaClO}	0.2 m^3/d
Pumping pressure	Pr_{chem}	2 kPa
Operating time	t	24 sa/d
Aluminum sulfate density	P_{alum}	2670 kg/m^3
Sodium hypochlorite density	P_{NaClO}	1110 kg/m^3
Rapid mixing		
Electricity consumption	$E_{rapid\ mixing}$	36 kWh
Slow mixing		
Electricity consumption	$E_{slow\ mixing}$	18 kWh
Backwasing		
Electricity consumption (for 3 motors)	$E_{backwashing}$	36 kWh
Other consumptions		
Building lighting and dosing pumps	E_{others}	9 kWh

On-site CO_2 emissions

On-site CO_2 emissions are mainly due to alkalinity production during chemical reactions in the mechanical mixing process of coagulants (aluminum sulfate) and buffer anions (CO_3^{2-} and HCO_3^-). Equation 1 shows the formation of CO_2 emissions due to the reaction of aluminum sulfate with calcium bicarbonate.

$$Al_2(SO_4)_3.18H_2O + 3Ca(HCO_3) \rightarrow 2Al(OH)_3 \downarrow + 3CaSO_4 + 6CO_2 + 18H_2O \quad (1)$$

To calculate on-site CO_2 emissions, the utilization of aluminum sulfate as a coagulant was considered, incorporating a CO_2 emission factor (EF_{chem}) based on stoichiometric mass balance. For aluminum sulfate, this factor is taken as 0.395 g CO_2e/g. Aluminum sulfate concentration was calculated by multiplying by the total flow rate to determine the total mass of aluminum sulfate used during the process. Daily CO_2 emissions from chemical reactions within the facility were calculated with Equation 2 (Kyung et al., 2013).

$$p_{co_2,chemical} = (C_{chem}\, x Q_{flow}\, x\, 10^3 Lm^{-3}\, x\, 10^{-6} kgmg^{-1}) x\, EF_{chem} \quad (2)$$

where is on-site CO_2 emission by chemical reaction (kg CO_2e/d), C_{chem} is concentration of coagulant (mg/L), Q_{flow} is flow rate (m^3/d), and EF_{chem} is CO_2 emission factor of coagulant (gCO_2e/g).

Off-site CO_2 emissions

CO_2 emissions related to electricity consumption

Off-site CO_2 emissions converted from electricity consumption used to operate the units in the drinking water treatment plant were calculated as given in Equation 3. Total emissions of the units were calculated using 0.7424 $kgCO_2e/kWh$ (TEIAS, 2023) as the CO_2 emission factor (EF_{elec}) for electricity consumption. In the calculations, chemical feed includes rapid and slow mixing and backwashing. Rapid mixing, slow mixing, and backwashing electrical consumption data were obtained from the drinking water treatment plant. Electricity consumption for sludge transport is not included in the calculations. Because the sludge of the drinking water treatment plant is discharged into the stream right below the facility. Calculations were made for electricity consumption and CO_2 emissions, assuming the efficiency of the pump, motor, and gear was 85%. Electricity consumption data of each unit was obtained from the drinking water treatment plant.

$$p_{co_2,electricity}=\sum[(E_{chemical\ supply}+E_{rapid\ mixing}+E_{slow\ mixing}+E_{backwashing}+E_{others})\ x\ EF_{elec}] \quad (3)$$

where is off-site CO_2 emission by electricity consumption (kg CO_2e/d), $E_{chemical\ supply}$ is energy consumption for chemical supply (kWh/d) $E_{rapid\ mixing}$ is energy consumption for rapid mixing (kWh/d), $E_{slow\ mixing}$ is energy consumption for slow mixing (kWh/d), $E_{backwashing}$ is energy consumption for backwashing (kWh/d) and EF_{Elect} is CO_2 emission factor for electricity generation (kgCO_2e/kWh).

Electricity consumption for chemical supply

Various chemicals are used to meet water quality standards during the operation of a drinking water treatment plant. Aluminum sulfate (Alum) coagulant was added during the rapid mixing process for effective floc formation. Sodium hypochlorite was added for disinfection. Off-site emissions during the production and transportation of chemicals used in drinking water treatment plants are calculated in Equation 4 (Kyung et al., 2013).

$$E_{chemical\ supply}=\sum\ (Pr_{chem}\ x\ Q_{chem}\ x\ t_{chem}\ x\ Eff_p^{-1}\ x\ 86400^{-1}) \quad (4)$$

Where $E_{chemical\ supply}$ is energy consumption for chemical supply (kWh/d), Pr_{chem} is chemical supply pump pressure (kPa), Q_{chem} is feed rate of chemical (m^3/d), t_{chem} is process operating time (hr/d), and Eff_p is efficiency of operating pump.

RESULTS AND DISCUSSION

On-site CO_2 emissions calculations

On-site CO_2 emissions occurred only due to alkalinity formation during chemical reactions in the mechanical mixing process of aluminum sulfate and buffer anions (CO_3^{2-} and HCO_3^-). The CO_2 emission factor for aluminum sulfate used in the drinking water treatment plant is 0.395 gCO_2e/g (Ecoinvent, 2019). Accordingly, when all the data were put into equation 2, the amount of CO_2e within the facility was 11.56 kgCO_2/d. Chemical selection in drinking water treatment plants is a factor that significantly affects the carbon footprint, especially for the coagulation unit. Generally, Fe coagulants have lower CO_2 emissions than Al coagulants. Emissions from producing Fe coagulants range from 29 to 395 kgCO_2e per ton, while Al coagulants range from 148 to 537 kgCO_2e per ton (INCOPA, 2014). This difference is due to factors such as the type of raw materials used (natural mineral or by-product), the processing method in the production process, and where the raw material is produced (Pellikainen et al., 2023).

Table 3. On-site CO_2 emissions due to chemical use.

Chemical	Dossage rate	Flow (m^3/d)	Emission factor[a] (kgCO_2e/kg)	CO_2e emission (kgCO_2e/d)
Aluminum sulfate	10	2928	0.95	11.56

[a]Ecoinvent 2019

Off-site CO_2 emissions calculations

CO_2 emission calculation based on off-site electricity consumption

Calculating CO_2 emissions from off-site electricity consumption requires a separate calculation for each unit, calculating chemical supply and then calculating total emissions. In this context, first, the CO_2 emissions of the chemical supply were calculated. The main energy consumption in chemical feed comes from the operation of injection pumps, and the total energy consumed by chemical feed is significantly affected by pumping pressure, feed rates of chemicals, operating time, and pump efficiency. Calculations were made for electricity consumption and CO_2 emissions, assuming the efficiency of the pump, motor, and gear was 85%. Accordingly, when the calculations related to the total chemical supply were made according to Equation 4, the electricity consumption was found to be $4.57x10^{-4}$ kWh. Rapid mixing, slow mixing, backwashing, and other off-site electricity consumption data are given in Table 4. As shown in Equation 3, by multiplying the total electricity consumption with the CO_2 emission factor for electricity consumption, 0.7424 kgCO_2e/kWh, the amount of CO_2e due to the total electricity consumption was calculated as 73.49 kgCO_2.

Table 4. Off-site CO_2 emissions due to electricity consumption.

Process	Electricity consumption	Emission factor[a] ($kgCO_2e/kWh$)	CO_2e emission ($kgCO_2e/d$)	Percentage (%)
Chemical supply	$4.57x10^{-4}$	0.7424	$3.40 x10^{-4}$	0.0004
Rapid mixing	36	0.7424	26.72	35.88
Slow mixing	18	0.7424	13.37	18.19
Backwashing	36	0.7424	26.72	35.88
Other consumption	9	0.7424	6.68	9.08

[a]T.C. Energy and Natural Resources Ministry, 2023

CO_2e emissions during drinking water treatment plant operation were calculated as in-site and off-site emissions, and each category's contribution percentage and CO_2e emissions are given in Table 5 and Figure 2.

Table 5. Total on-site and off-site CO_2e emissions of the drinking water treatment plant and percentage distribution of each category.

Emission type	Category	Emission ($kgCO_2e/d$)	Percentage (%)
In-site CO_2 emissions	Chemical reaction	11.56	13.6
Off-site CO_2 emissions	Electricty consumption	73.49	86.4
Total CO_2 emissions		85.05	100

The results show that the most significant CO_2 emissions come from electricity consumed at the facility. The electrical energy used in the units releases 73.49 $kgCO_2e/d$ of CO_2 outside the facility, constituting 86.4% of the total value. In emissions related to electricity consumption, the highest CO_2 release occurred in rapid mixing and backwashing to treat raw water with a flow rate of 2928 m^3/d. During the rapid mixing and filtration unit backwashing process, 26.72 $kgCO_2e/d$ emissions related to electricity consumption occurred for each unit. This is thought to be due to the mechanical mixing of impellers with high rotation speed to maintain the appropriate speed gradient for the rapid mixing process, while for the backwash process, it is due to the electrical energy used by the backwash pump and air blowers. CO_2 release from electricity consumption in the slow mixing process is 13.37 $kgCO_2/d$. Finally, CO_2 emissions due to off-site electricity consumption arise from electricity consumption in the chemical supply process. The contribution to CO_2 emissions at this stage is 3.40×10^{-4} $kgCO_2/d$ and is negligible. This is because the pumps feeding the chemicals are operated at very low pressure compared to other processes and consume less electrical energy.

Upon investigation of on-site CO_2 emissions, it was found that the CO_2 emission attributed to the chemical reaction during this stage amounted to 11.56 $kgCO_2/d$. When aluminum sulfate is used as a coagulant, on-site CO_2 emissions from chemical reactions constitute a small portion (13.6%) of the total on-site and off-site CO_2 emissions. It has been determined that CO_2 production in the aqueous phase based on stoichiometric mass balance is directly related to the consumption of buffer anions. Different coagulant substances (powdered activated carbon, iron sulfate, etc.) can be tried to reduce the CO_2 emissions resulting from the chemical reaction (Zamfiroiu & Masu, 2007). In this way, it is thought that CO_2 emissions can be reduced with advantages such as less sludge production and less use of chemicals. Although on-site CO_2 emissions are very small, CO_2 emissions can be significantly reduced by low alkalinity consumption and appropriate coagulation dosages to reduce on-site emissions in drinking water treatment plants (Pellikainen et al., 2023).

When the literature is examined, there are few studies on the carbon footprint calculation of drinking water treatment plants (Yateh et al., 2024). There are studies mostly aimed at calculating the carbon footprint of wastewater treatment plants. Nevertheless, there are studies in the literature to reduce the carbon emissions of drinking water treatment facilities. One of them, Kyung et al. (2013), is their study. This study compared the carbon emissions of classical and advanced drinking water treatment plants. Carbon emissions were found to be higher in the advanced drinking water treatment plant because the membrane and ozonation unit are additional processes. Similar to the presented study, off-site carbon emissions were found to be higher in both drinking water treatment plants. However, the carbon footprint of the classical wastewater treatment plant was higher than that of the presented study. One of the most important reasons is that the flow rate to the facility is very high. Beeftink et al. (2021)estimated carbon emissions for drinking water treatment plants using the softening method and found that chemical consumption increased electrical energy consumption. Maziotis et al. (2023) calculated in another study that the total drinking water

emissions associated with electricity consumption across the USA were 26.5×10^9 kgCO_2e. They found that water and wastewater electricity generation contributes 2% of total greenhouse gas emissions each year.

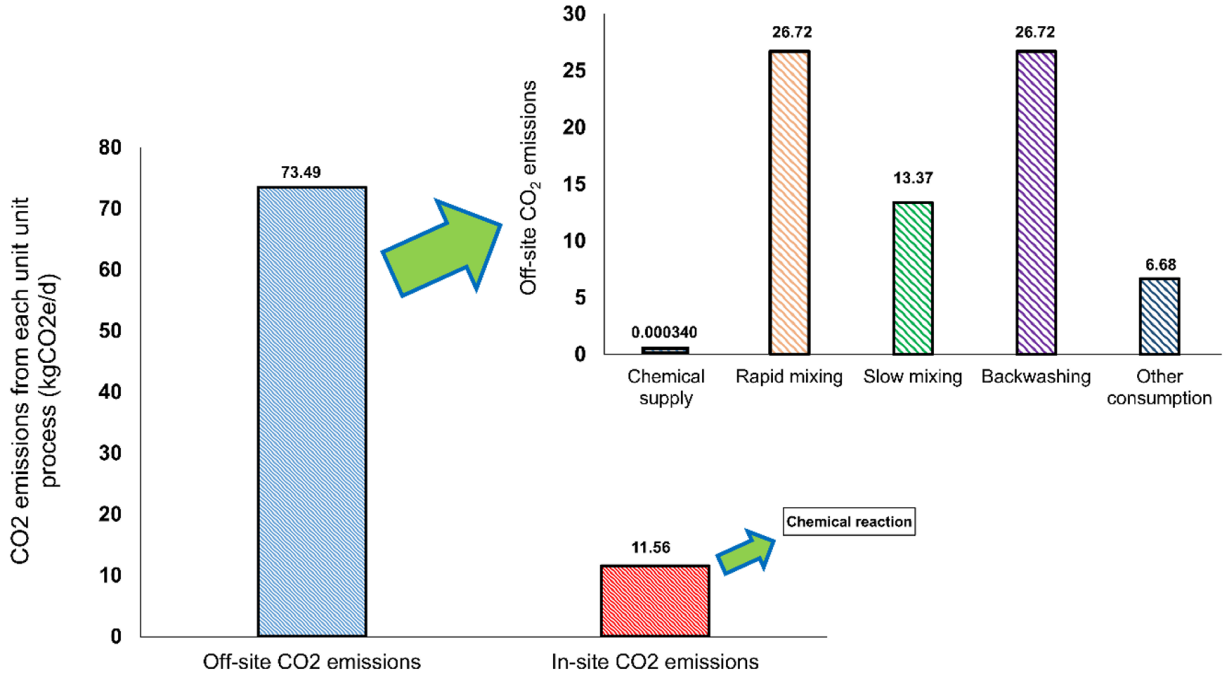

Figure 2. The CO_2 emissions from each unit process of the drinking water treatment plant

CONCLUSION

This study calculated the carbon footprint of the drinking water treatment facility within Samsun Ondokuz Mayıs University, which supplies clean water to the campus. In this context, the drinking water treatment plant's on-site and off-site CO_2 emissions were calculated. Accordingly, the total CO_2e emission of the drinking water treatment plant is 85.05 kg CO_2e per day, and the annual amount is estimated to be approximately 31.04 tons CO_2e. In the study, it was determined that the facility's primary source of CO_2 emissions occurred during electrical consumption. To reduce carbon emissions caused by electricity consumption, air blowers, pumps, etc., consume less electricity, and it is recommended to use equipment. Switching to electricity production from renewable sources (wind, solar, biomass, etc.) will help reduce carbon emissions. Within the scope of the European Union Green Deal, Turkey aims to reduce greenhouse gas emissions by 55% in 2030 and become carbon neutral in 2050. In this context, preventing and reducing greenhouse gas emissions from drinking water and wastewater treatment facilities is important. Treatment plants must pursue new green and sustainable water treatment technologies with high pollutant removal efficiency and low CO_2 emissions. Considering greenhouse gas emission issues in the water treatment sector is important for understanding the relationship between water quality and greenhouse gases. This study is thought to be a good example of preventing greenhouse gas emissions in public regulations regarding authorities' treatment facilities.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author has declared no conflict of interest.

Author contribution

Sevde Üstün Odabaşı: conceptualization; investigation; methodology; data curation funding acquisition; writing-review & editing.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Data available on request from the authors.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Effects of mycorrhizal fungi application on some growth parameters of Monterey strawberry cultivars under different salt stress conditions

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Abstract
Salinity is one of the most important environmental problems for agricultural production. In recent years, some studies revealed that arbuscular mycorrhizal fungi positively influenced plant growth and development and increased the uptake of nutrients under saline conditions. This study was carried out to investigate the response of the Monterey strawberry cultivar to mycorrhizal fungi (*Glomus spp.*) root inoculations during salinity stress under greenhouse conditions. In the present study, four different salt concentrations (0, 20, 40, and 80 mM NaCl) were applied to growing media with and without mycorrhiza conditions. The parameters such as leaf number, leaf area, petiole length, root length, dry matter contents in leaves, crowns, and roots, fruit weight, fruit yield, and Na, P, and K accumulation in leaves, crowns, and roots were determined. It was determined that increasing the amount of salt negatively affected all parameters in the control treatment. Leaf area significantly decreased with increasing concentration of NaCl treatment with and without mycorrhiza conditions. High salinity caused an increase in the Na content, but K and P contents decreased with salinity. Finally, it is suggested that the application of mycorrhizae generally positively affected examined parameters in strawberries under salinity conditions.
Keywords: Strawberry, Salt stress, Arbuscular Mycorrhizal Fungi, Growth parameters, Yield

INTRODUCTION

Strawberries (*Fragaria ananassa*), belonging to the Rosaceae family, are sweet-scented, fleshy, and juicy false fruits (Zhang et al., 2011). Strawberries are considered a functional food due to their biological activities and potential health benefits, and they are widely consumed (Siebeneichler et al., 2022).

In recent years, strawberry cultivation has spread to almost all regions of Turkey. The total annual production has now reached 728.112 tons (TUIK, 2023). With the increase in strawberry production, stress factors such as salinity, which limit cultivation, have emerged. In recent years, researchers have been focusing on the selection of salt-resistant or salt-tolerant varieties since the problem of soil salinity is difficult and expensive to eliminate (Kamar et al., 2023).

Strawberry plants are considered to be a salt-sensitive crop and it was reported that salt stress conditions negatively affect plant growth and yield (Saied et al., 2005; Karlıdağ et al., 2009; Yıldırım et al., 2009). In strawberry cultivation, using poor-quality water for fertilization and irrigation purposes also causes a gradual accumulation of salt in the soil (Jamalian et al., 2013).

Plants are frequently exposed to adverse environmental conditions such as

drought, salinity, and cold stress during their growth and development (Gómez-del-Campo 2015; Azizinya 2005). It is estimated that about one-third of the world's cultivated land is affected by salinity (Kaya et al., 2002). In Turkey, these conditions adversely affect approximately 1.5 million hectares (Dinc et al., 1993). Salinity has complex effects on plants, and the adverse effects of salinity are associated with water deficit, ionic imbalance, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon allocation, and utilization (Bohnert et al., 1996; Moghaieb et al., 2001). Salinity stress negatively affects plant physiology both the whole plant and at cellular levels through osmotic and ionic stress. High salt concentrations in the soil can negatively affect seed germination, growth, flowering, and fruit set, leading to decreased crop yield and quality (Arora et al. 2008).

About a century ago, Frank, a German scientist (forest phytopathologist), made a major discovery regarding plant-fungal interactions. He identified fungi that were abundant in the roots of some plants but did not cause infection. In 1885, he named these fungi "mycorrhiza" (myko+rhiza), a combination of the words "fungus" and "root". Although this symbiotic relationship between plants and soil microorganisms is the most common, most important, and most interesting one of all symbiotic relationships (Allen, 1991), it recently began to gain importance, and the number of studies on it has been increasing (Allen, 1991; Gai et al., 2006; Martin and Slater, 2007).

The most effective mycorrhizal fungi in horticultural crops are vesicular-arbuscular mycorrhizas (VAM) (Rouphael et al., 2015). The utilization of symbiotic microorganisms that promote plant growth, especially arbuscular mycorrhizal (AM) fungi, can be useful in the development of strategies to facilitate plant growth in saline soils (Younesi and Moradi, 2014). The benefits that mycorrhizal fungi offer to plants include the provision of growth-promoting substances (auxin, cytokinin, and gibberellin, etc.), increased resistance to toxic substances left by heavy metals, increased resistance of seedlings to drought, and contribution to the control of root diseases in plants (Palta et al., 2010).

Many studies showed that inoculation with AM fungi improves the growth of plants under salt stress (Sharifi et al., 2007). The improved growth of AM plants was attributed to their enhanced ability to take up important nutrient elements such as N and P (Jeffries et al., 2003). However, in some cases, the plant's salt tolerance was not related to P concentration (Ruiz-Lozano and Azcón 2000). Thus, it was argued that salt-tolerance mechanisms, such as enhanced osmotic adjustment and reduced oxidative damage or improved nutritional status, can explain the contribution of AM symbioses to the salinity resistance of host plants (Augé 2001). The salt tolerance of plants may not be related to the amount of phosphorus (P) under certain conditions (Ruiz-Lozano and Azcón 2000). It was emphasized that arbuscular mycorrhizal (AM) symbioses increase the resistance of plants to salinity and that this is achieved by osmotic adjustment and reduced oxidative damage or improved nutritional status (Augé 2001).

The present study aims to investigate the effect of Mycorrhiza fungus on some growth and development parameters of Monterey strawberry cultivar grown at different salt concentrations.

MATERIALS AND METHODS

This study was carried out in a plastic greenhouse of the Department of Plant and Animal Production in the Suluova Vocational School of Amasya University in 2016. The 'Monterey', which is a neutral day strawberry variety, was used in this study. A commercial preparation containing Glomus spp. fungal species (*Glomus intraradices, Glomus aggregatum, Glomus mosseage, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum, and Gigaspora margarita*) was used for mycorrhiza inoculation.

Trial Design and Method

The trial was set up in a randomized block design with 3 replications and 15 plants per replication. In the study, frigo strawberry seedlings were planted in plastic pots (22x40) on 04.15.2016. Two different media were used as growing media. The first consisted of 100% peat and the second consisted of a mixture of 50% garden soil and 50% peat. Mycorrhiza application was performed immediately before planting. The seedlings were dipped in a solution prepared by mixing 250 g of the powdered preparation with 10 liters of water for 30 seconds, thus ensuring the inoculation of the roots with the fungus.

The nutrient solution was not initially supplemented with salt (NaCl), but the salt treatment was started when the seedlings had 4-5 leaves old after a 40-day development period. Salt applications were applied weekly four times with 100 ml of nutrient solution containing a salt solution of 0, 20, 40, and 80 mM/L NaCl. Plants were irrigated periodically, and their nutrient requirements were met by making use of a nutrient solution (Hoagland and Arnon. 1938) at regular intervals. Control plants were given a normal nutrient solution without salt application. The growth of strawberry plants was monitored after salt application, and they were removed in October after 6 months. The applications were given in groups in the trial. The descriptions of the groups are given below (Table 1).

Table 1. NaCl doses, media conditions, and mycorrhiza inoculation applications

Groups	Description
S_0PNM	Nonmycorrhizal application in peat media without salt application
S_0PM	Mycorrhizal application in peat media without salt application
S_0PGNM	Nonmycorrhizal application in peat x garden soil media without salt application
S_0PGM	Mycorrhizal application in peat x garden soil media without salt application
S_{20}PNM	Nonmycorrhizal application in peat media with 20 mM/L salt application
S_{20}PM	Mycorrhizal application in peat media with 20 mM/L salt application
S_{20}PGNM	Nonmycorrhizal application in peat x garden soil media with 20 mM/L salt application
S_{20}PGM	Mycorrhizal application in peat x garden soil media with 20 mM/L salt application
S_{40}PNM	Nonmycorrhizal application in peat media with 40 mM/L salt application
S_{40}PM	Mycorrhizal application in peat media with 40 mM/L salt application
S_{40}PGNM	Nonmycorrhizal application in peat x garden soil media with 40 mM/L salt application
S_{40}PGM	Mycorrhizal application in peat x garden soil media with 40 mM/L salt application
S_{80}PNM	Nonmycorrhizal application in peat media with 80 mM/L salt application
S_{80}PM	Mycorrhizal application in peat media with 80 mM/L salt application
S_{80}PGNM	Nonmycorrhizal application in peat x garden soil media with 80 mM/L salt application
S_{80}PGM	Mycorrhizal application in peat x garden soil media with 80 mM/L salt application

Growth analysis

After harvesting, the plants were washed using plenty of water and passed through pure water. After the moisture was completely removed by using a drying paper, the root length (cm) and leaf petiole length (cm) were measured using a ruler. The number of developed leaves on each plant was counted individually. The surface areas of the leaves were determined using a planimeter (ADC BioScientific Area Meter AM300) with a measurement unit of cm^2 (Demirsoy et al., 2005). After harvesting, the plants were separated into roots, crowns, and leaves. They were then placed in paper bags and kept in an oven at 70°C until their weights did not change. Their dry weights (g) were then determined by weighing. The weighing process was carried out on a sensitive balance sensitive to 0.001 grams. The average fruit weight was determined by weighing the fruits in each treatment and dividing by the number of fruits. The fruits belonging to the plants in each treatment were harvested periodically, then they were weighed, and the yield amount (g/$plant^{-1}$) was determined by dividing by the number of plants. The harvested fruits were weighed on a balance sensitive with 0.1g sensitivity (Karaduva, 1992).

Determination of P, K, and Na (%)

In the laboratory, dried leaf, crown, and root samples were ground to pass through a 1-mm sieve and prepared for analysis. Phosphorus content was determined by using the vanadomolybdophosphoric yellow color method using a Thermo Scientific (Genesys 10S UV-VIS) spectrophotometer at a wavelength of 430 nm (Kacar, 1991). Potassium and sodium contents were determined by using atomic absorption spectrophotometry (Thermo Scientific (ICE 3000 Series)) in a solution obtained by wet ashing with a mixture of nitric and perchloric acids in root, crown, and leaf samples dried at 70°C in an oven (Kacar, 1972; 1991).

Statistical Analysis

The present study was carried out by using a randomized block design with 3 replications and 15 plants per replication. The statistical analyses of the results (SPSS 20.0) were conducted by performing a one-way analysis of variance. Multiple comparisons of the means were performed at the 5% significance level by using Duncan's multiple comparison method.

RESULTS AND DISCUSSION

The effects of four different concentrations of NaCl (0, 20, 40, and 80 mM/L), two different media (peat + garden soil), and mycorrhizal application on some growth parameters and yield of Monterey strawberries were investigated. The results obtained from this study are discussed below. The statistical analysis results of the data obtained in this study (root length, petiole length, leaf number, leaf area, dry weights, fruit weight, yield, and P, K, and Na contents) are given in Table 2.

Root Lenght

In this study, the highest root length (42.39 cm in S0PGM) was obtained from plants treated with mycorrhiza in a peat x garden soil medium without salt application (Table 2). The lowest root length value was 24.58 cm (S80PGNM), again obtained from plants treated with 80 mM/L salt without mycorrhiza in a peat x garden soil medium. The positive effect of mycorrhiza applications on root length was found to be statistically significant in both media. The results obtained show that root length decreases significantly with increasing salt concentration. Similarly to the present study, a study carried out in vitro on strawberries revealed that increasing salt concentration caused decreases in root length, root number, shoot number, and plant height (Erenoğlu et al., 2003). Another study was carried out on the effects of EC levels on yield and quality in the Camarosa strawberries by using different growing media (peat and coir) and it was determined that EC levels significantly affected plant growth and development when compared to growing media (Adak, 2010). In a study that compared the effects of bacterial and mycorrhizal applications under different salt stress conditions (0, 30, and 60 mM/L NaCl) on the San Andreas strawberry variety, the effects of salt concentrations and harvest days on root length were found to be statistically significant, whereas the effects of applications were found to be insignificant (Koç et al., 2015). Similarly to the present study, it was determined that increasing salt concentration caused a decrease in root length in Boysenberry plants to which only NaCl concentrations were applied (Kurt, 2021).

Leaf Petioles Length

In the experiment, examining the applications for petiole length, the highest value was determined to be 14.10 cm obtained from the S0SGM application, whereas the lowest value was found to be 8.55 cm obtained from the S80SNM application (Table 2). Petiole length decreased significantly with increasing salt concentrations. The effect of mycorrhiza application was generally evident in all applications. Comparing the mycorrhizal-applied media, it was determined that petiole length was higher in the peat x garden soil medium. Similar to the present study, Sönmez et al. (2013) found in their study investigating the effects of zinc, salt, and mycorrhiza on corn development that mycorrhizal application yielded the highest value in plant height and that plant height decreased under saline conditions. Pirlak and Eşitgen (2004) reported in their study that the length of strawberries' vegetative parts did not change with increasing salt application.

Leaf Number

In the present study, leaf numbers of the Monterey strawberry variety subjected to increasing NaCl applications are shown in Table 2. The highest leaf number was obtained with the S0PGM application with 12.99 leaves, whereas the lowest value was obtained with the S80PNM application with 8.87 leaves. The number of leaves decreased significantly with increasing salt concentration. The effect of mycorrhizal application on leaf number in peat media was found to be statistically significant as the salt concentration increased. Strawberries are known to be sensitive to salt stress and a study reported a decrease in stolon length, stolon number, leaf number, and fresh and dry root weights (Saied et al., 2005). In their study, Keutgen and Pawelzik (2009) found that the leaf numbers of the Korono and Elsanta strawberry varieties were 29.20 (control), 15.80 (40 mmolL^{-1}), and 11.90 (80 mmolL^{-1}) and 16.30 (control), 9.50 (40 mmolL^{-1}), and 8.20 (80 mmolL^{-1}), respectively. Similarly to the present study, it was found that the leaf number decreased significantly as the salt concentrations increased. Pirlak and Eşitgen (2004) applied three concentrations of salt (2.0, 5.0, and 7.5 mS cm^{-1} EC) to strawberries and reported that the leaf number of strawberries was negatively affected by salinity. In their study, the leaf number decreased from 37.74% to 29.79% as the salt level increased from 2.0 to 5.0 mS cm^{-1} EC.

Leaf Area

The results of the statistical analysis conducted to determine the effect of different salt doses and mycorrhizal application on the leaf area of strawberries grown in different media are shown in Table 2. In the experiment, the highest leaf area was obtained from the control applications of 42.00 cm^2 (S0PM) and 41.65 cm^2 (S0PGM), while the lowest values were obtained from the applications where the salt concentration was 80 mM/L, with values of 26.61 cm^2 (S80PNM), 24.94 cm^2 (S80PM), and 25.48 cm^2 (S80PGM). In the experiment, it was determined that the leaf area decreased significantly as the salt concentrations increased. In addition, it was found that the leaf area in mycorrhizal application was generally higher than others.

In their study, Keutgen and Pawelzik (2009) found that the leaf area of the Elsanta strawberry variety decreased due to the increase in salinity. They reported that this was due to the increase in Na^+ and Cl^- ions in the root region and the appearance of typical symptoms on strawberry leaves. They also stated that necroses, which turn from red to brown starting from the leaf tips and spreading to the edges, occur at advanced stages of salinity and even lead to death. Similarly, in the experiments conducted with the Monterey strawberry variety in the present study, the necrosis caused by the increase in Na^+ and Cl^- concentrations in the root region covered the entire leaf surface and resulted in a significant decrease in leaf area. Üzal and Yıldız (2014) found that the effect of high salt concentrations on strawberry plants is first seen as symptoms such as yellowing and necrosis of old leaves. Over time, this effect manifests itself

as decreases in biomass weight and leaf area, particularly in the leaves of the plants. Keutgen and Pawelzik (2009) stated that the decrease in leaf area in both strawberry varieties they used was due to a decrease in the number of leaves. In their study with the Albion, Benicia, Monterey, San Andreas, and Ventana strawberry varieties, Ferreira et al. (2019) applied salt at doses of 0.7 (control), 1.0, 1.5, and 2.5 dS m^{-1} for 240 days and determined the survival rates of the varieties. Albion had the highest survival rate (94%) at the highest salt concentration (2.5 dS/m), followed by San Andreas (77%), Benicia (75%), and Ventana (67%). Monterey had the lowest survival rate (53%) at the 2.5 dS m^{-1} salt application.

Dry Weights

In the Monterey strawberry variety subjected to NaCl stress, the dry weights measured at the end of the 6-month growing period are given in Table 2. The difference between the salt and control applications was found to be significant in terms of the dry leaf, dry crown, and dry root weights measured in the experiment.

In the experiment, the highest dry leaf weight was obtained from the control group without salt application, 19.08 g/plant (S0PGM). The lowest dry leaf weight in the experiment was determined in the applications where the salt dose was 80 mM/L, 8.81 g/plant (S80PNM). Leaf dry weights decreased significantly as salt concentrations increased. The positive effect of mycorrhiza was statistically significant in the applications where the salt dose was 40 and 80 mM/L.

In the experiment, the S0PM application with mycorrhiza inoculation yielded the highest crown dry matter content, with an average value of 4.21 g/plant, whereas the S0PGM application with mycorrhiza application had an average of 4.24 g/plant. The S80PNM application without mycorrhiza had the lowest crown dry matter content, with an average of 1.96 g/plant, and the S80PGNM application without mycorrhiza had an average value of 2.18 g/plant. Examining the dry crown weights, the effect of mycorrhiza application was found to be statistically significant at high salt concentrations.

The root weights of the plants in peat x soil medium without salt stress and with mycorrhiza inoculation reached the highest dry matter ratio with 4.29 g/plant and 4.27 g/plant in peat medium, while the plants in peat medium containing 80 mMol salt without mycorrhiza were determined to have the lowest values with an average of 2.00 g/plant. It was found statistically significant that root dry weights decreased with increasing salt levels like other growth parameters. The positive effect of mycorrhiza on root dry weights was statistically significant at 40 and 80 mM/L salt doses.

Üzal and Yıldız (2014) reported that leaves were affected the most by increasing salt stress on strawberries. They found that leaf weight and leaf area decreased significantly when compared to other parameters in the experiment and that the level of decrease increased as the application duration increased. It was reported that this decrease varies depending on the variety. It was concluded that the entire plant was not negatively affected as a result of accumulating Cl^- ions in the root region in some varieties (Saied et al., 2003). In another study, it was determined that the dry leaf weight of the Camarosa and Tioga strawberry varieties decreased with increasing salt concentration (Turhan and Eriş, 2005). It was determined that the effects that started with necrosis on grape leaves continued to increase as the salt concentrations increased (Sivritepe, 1995).

Yılmaz and Kına (2008) showed in their studies that the dry crown weights of the Gloria variety exhibited a general increasing trend, unlike the present study. Kına (2008) reported that the fresh and dry root weights of the Kabarla variety increased with increasing salt applications, but there was a slight decrease in the Gloria variety. Turhan and Eriş (2005) found an increase in the early stages of salt applications in the Camarosa variety, followed by a decrease. Chartzoulakis and Klapaki (2000) reported a significant decrease in leaf, crown, and root dry weights of pepper as salt stress increased. In a study carried out on Kent, Jewel, and Saint-Pierre strawberry varieties, three different salt doses were applied (0, 30, and 60 mM/L), and it was determined that the fresh weight of leaves and roots decreased as the salt doses increased, whereas both leaf and root fresh weights were higher in mycorrhizal-inoculated plants. Keutgen and Pawelzik (2009) found a decrease in the growth of the Camarosa strawberry variety in the application where the salt was 40 mg NaCl.

Fruit Weight

The effects of mycorrhiza on the average fruit weight per plant in this study, where different salt doses were applied to two different environments, are given in Table 2. In the experiments, it was found that the effect of mycorrhiza application on the average fruit weight per plant was significant at the $p < 0.05$ level under increasing salt stress. In this study, the highest average fruit weight was obtained from the S0PNM application (4.72 g), whereas the lowest average fruit weight was obtained from the S80PNM application (2.52 g). The effect of mycorrhiza on the average fruit weight is significantly evident, especially in applications with 80 mM/L concentration. The increase in salt concentrations caused statistically significant decreases in the average fruit weight. The effect of the environmental

difference on the average fruit weight was unclear.

Keutgen and Pawelzik (2008) found that salt application reduced the average fruit weight of the Elsanta strawberry variety. At the 80 mM/L NaCl concentration, 26% and 46% decreases in fruit weights were found in Korona and Elsanta varieties, respectively. Sato et al. (2006) related the decrease in fruit size in environments with increasing salt concentrations to the inhibition of water uptake and the decrease in water transport to the fruit. In parallel with the present study, Kamar et al. (2023) determined the highest fruit weight value in the control group in their study with 4 different salt concentrations (1 dS/m, 1.5 dS/m, 2 dS/m, and 2.5 dS/m) and 3 strawberry genotypes, and they found that the average fruit weight values decreased with increasing salt dose.

Yield

The effects of mycorrhiza application on average fruit yield per plant in different environments under long-term salt stress are presented in Table 2. In this study, it was found that the effect of mycorrhiza application on the average fruit yield of strawberries under increasing salt stress was statistically significant. The lowest average fruit yield per plant was obtained from the S80PNM application (61.20 g) with a salt dose of 80 mM/L, whereas the highest value was obtained from the S0PM and S20PGM applications with 99.63 g and 99.17 g, respectively. In general, the mycorrhiza application yielded positive results in the fruit yield of the Monterey strawberry variety under salt stress conditions.

As a result of the studies on salt stress, it was determined that root, crown, and shoot development and fruit weights and yields of plants decreased and fruit quality was also negatively affected (Abbas et al., 1991; Franco et al., 1993). In a study carried out with Elsanta and Korono strawberry varieties, it was found that fruit yield and number of fruits per plant were not affected by salinity in the first year but decreased significantly in the second year (Saied et al., 2005). Ferreira et al. (2019) carried out a study with Albion, Benicia, Monterey, San Andreas, and Ventana strawberry varieties by using a salt tolerance modeling approach based on all reductions in dry weight and fruit yield, and they determined that Albion was the most tolerant, whereas San Andreas and Ventana were sensitive, and Benicia and Monterey were moderately tolerant. Kamar et al. (2023) observed that different salt concentrations negatively affected yield in the No. 112 and No. 36 strawberry genotypes, with the greatest yield reductions occurring in the Fortuna strawberry variety.

Table 2. Effect of inoculation with mycorrhizal fungi on root length, petiole length. leaf number, leaf area, dry weights, fruit weight, and yield of Monterey strawberry grown in two media at four salinity levels.

Application	Root length (cm)	Petiole length (cm)	Leaf number (number)	Leaf area (cm^2)	Dry leaf weight (g)	Dry crown weight (g)	Dry root weight (g)	Fruit weight (g)	Yield (g)
S_0PNM	39.90 abc	12.37 c	12.02 abcd	40.08 ab	18.46 a	4.14ab	3.94 ab	4.72 a	87.10 abc
S_0PM	42.02 ab	13.57 ab	12.33 abc	42.00 a	18.97 a	4.21 a	4.27 a	4.43 ab	99.63 a
S_0PGNM	38.98 bc	12.49 bc	12.55 ab	39.6 4abc	18.22 a	3.94 abc	3.97 ab	4.25 abc	89.13 abc
S_0PGM	42.39 a	14.10 a	12.9 9a	41.6 5a	19.08 a	4.24 a	4.29 a	4.50 ab	92.66 ab
S_{20}PNM	37.42 cd	11.61 cde	11.56 bcde	36.99 bc	15.95 b	3.42 d	3.89 ab	3.45 abcd	75.33 abc
S_{20}PM	37.75 cd	11.88 cd	11.00 defg	37.99 bc	16.59 b	3.61 cd	3.76 b	4.51 ab	85.57 abc
S_{20}PGNM	34.92 de	12.04 cd	12.04 abcd	36.63 c	15.98 b	3.29 de	3.80 b	4.34 ab	82.08 abc
S_{20}PGM	38.05 cd	12.31 c	11.35 cdef	38.09 bc	18.13 a	3.70 bcd	3.94 ab	4.44 ab	99.17 a
S_{40}PNM	37.34 cd	10.51 efg	10.60 efgh	30.72 de	12.09 d	2.87 e	2.69 d	3.63 abcde	71.40 abc
S_{40}PM	36.70 cd	10.01 fg	11.11 defg	30.01 de	13.78 c	3.54 cd	3.37 c	3.61 abcde	74.80 abc
S_{40}PGNM	28.23 ef	11.51 cde	11.02 defg	30.17 de	12.24 d	2.88 e	2.77 d	4.04 abc	65.27 bc
S_{40}PGM	36.48 de	10.94 def	10.92 efgh	32.72 d	14.06 c	3.34 d	3.75 b	3.68 abcd	71.97 abc
S_{80}PNM	27.99 f	8.55 gh	8.87 hı	26.61 f	8.81 f	1.96 f	2.00 f	2.52 ef	61.20 c
S_{80}PM	33.80 e	9.69 fg	9.80 h	24.94 f	10.33 ef	2.88 e	2.39 de	3.09 cd	68.17 bc
S_{80}PGNM	24.58 g	9.27 g	10.30 fgh	27.47 ef	9.06 f	2.18 f	2.14 ef	2.75 e	66.05 bc
S_{80}PGM	36.52 de	9.98 fg	10.05 gh	25.48 f	11.12 de	2.85 e	2.73 d	3.14 bcd	71.86 abc

$p<0.005$

% P content

The effects of mycorrhiza inoculation on strawberry plants on P contents of the roots, crowns, and leaves under different salt treatments and media are illustrated in Figure 1. Increasing salt levels significantly reduced the average P accumulation in strawberries. In the present study, the highest average P accumulations in leaves, crowns, and roots were determined to be 0.52% (S0PGM), 0.44% (S0PGM), and 0.31 % (S0PM), respectively, whereas the lowest values were found to be 0.14% (S80PGNM), 0.14% (S80PM), and 0.11% (S80PM). The effect of the media on P concentration was found to be not significantly observed, whereas the average P content of roots, crowns, and leaves was statistically significantly lower at high salt levels than in the control. Comparing the P contents of plant parts at the same salt concentrations, the effect of mycorrhiza inoculation was found to be significant.

In parallel with the present study, Pirlak and Eşitgen (2004) reported that N, P, K, Na, and Cl ions were significantly affected by salt applications. They also found that K and P contents in plant leaves were higher at low salt levels than at high salt levels. Moreover, Erdal et al. (2000) examining cucumber seedlings and Alpaslan et al. (1998) examining wheat found that P uptake was negatively affected under increasing salt conditions and P content in the plant decreased. Sönmez et al. (2013) applied two different salt doses (0 and 100 mg Na Cl/kg) to maize plants and reported a 29% increase in P content. Similarly, Güneş et al. (1999) reported that P uptake increased with increasing salt concentration. They stated that this may be due to the increase in the available P content of the soil in saline environments and the synergistic effect of Na. In another study, it was reported that salinity limited the uptake of P and other elements in particular (Evelin et al., 2009).

% K content

The changes in K content in roots, crowns, and leaves of strawberries after mycorrhiza application at different salt levels and in different media are given in Figure 2. In this study, the highest and lowest K accumulation values were determined to be 3.24% (S0PGM), 1.15% (S80PGNM), and 1.18% (S80PNM) in leaves, 2.89% (S0PNM), 1.15% (S80PNM), and 1.17% (S80PGNM) in crowns, and 3.03% (S0PGM) and 0.52% (S80PNM) in roots. It was found that the K content decreased significantly with increasing salt doses applied to leaves, crowns, and roots. Additionally, the effect of mycorrhiza resulted in increased K accumulation in all organs.

Keutgen and Pawelzik (2009) reported that K content varies among the organs of strawberries. In their study, K content was determined to be the highest in the petiole and the lowest in the roots in both cultivars and treatments. In Elsanta, K levels increased in fruit and petioles, whereas Korono showed an increase in leaves and crowns. Essa (2002) reported that high Na uptake affects the uptake of elements such as K^+, Ca^{+2}, and Mg^+ in plants such as soybeans. Salt-tolerant cultivars had lower concentrations of Na^+ and Cl^- in their leaves, while K^+ concentrations were high. In contrast, Ferreira et al. (2019) did not observe an increase in Na^+ in leaves or competition between Na^+ and K^+ in cultivars. The high levels of Na and Cl in the soil can inhibit the uptake of K, Ca, and N ions in plants and also disrupt the ionic balance of plants (Güneş et al., 1999; Botella et al., 2005; Hong et al., 2009).

% Na content

The changes in Na ion accumulation percentages in root, crown, and leaf by different salt treatments are illustrated in Figure 3. In the present study, the highest and lowest Na accumulation levels were determined to be 4.01% in S80PGNM, 3.93% in S80PGM, and 0.79% in S0PNM in the leaves, 4.67% in S80PGM, 4.41% in S80PGNM, and 1.01% in S0PM in the crowns, and 5.01% in S80PGNM, 0.99% in S0PNM, and 1.04% in S0PM in the roots. In contrast to P and K levels, Na levels in leaves, crowns, and roots increased significantly with increasing salt doses. In general, Na content in leaves, crowns, and roots was lower in mycorrhiza treatments when compared to others.

Keutgen and Pawelzik (2009) reported that the presence of NaCl in the root zone of strawberries caused a decrease in N and Cl concentrations in all organs of strawberries. In their study, an increase in Na accumulation was observed in the fruit, leaves, petioles, crowns, and roots of the Korono and Elsanta strawberry varieties. Ferreira et al. (2019) reported in their study on 5 strawberry varieties that the salt did not affect the Na^+ concentration in the leaves of all varieties, but it affected both the roots and petioles, depending on the variety. Rahimi and Biglarifard (2011) stated that the high Na^+ level in the roots and crowns of the Camarosa variety was due to the higher proportion of dry weight of the roots and crowns. Pirlak and Eşitken (2004) found that the accumulation of Na and Cl in the leaves of both Camarosa and Fern varieties increased with increasing salinity. Marschner (1995) reported that plants take up more Na and Cl in saline environments. Again, Pirlak and Eşitken (2004) found that increasing the NaCl concentration in the nutrient solution increased the Na and Cl concentrations in the leaves. Moreover, Na and Cl ions accumulated more in both varieties at the 5.0 mS cm^{-1} EC salt level when compared to the 2.0 mS cm^{-1} EC level.

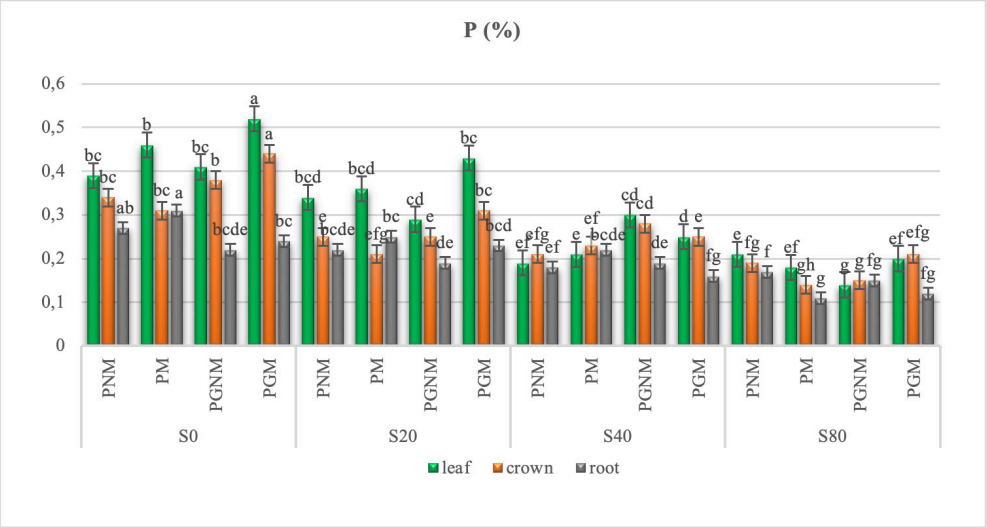

Figure 1. % P contents of the treatments

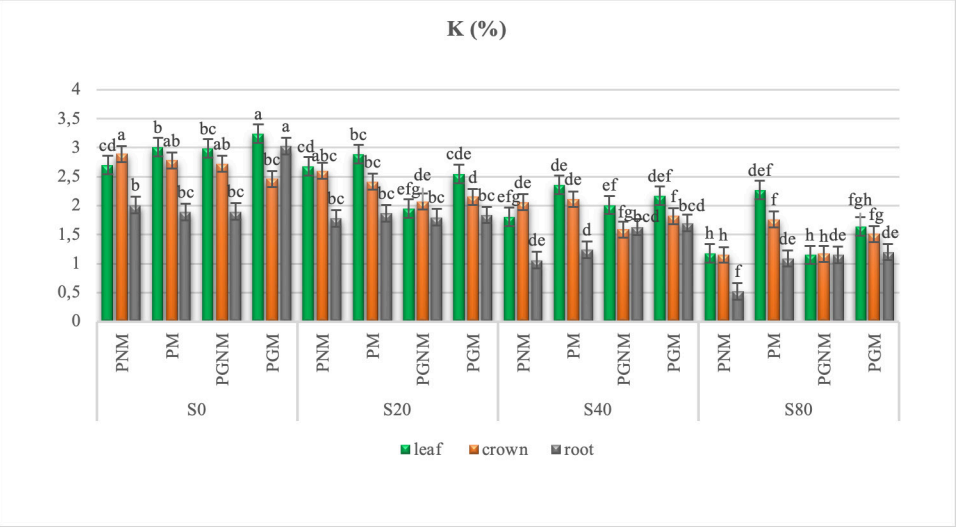

Figure 2. % K contents of the treatments

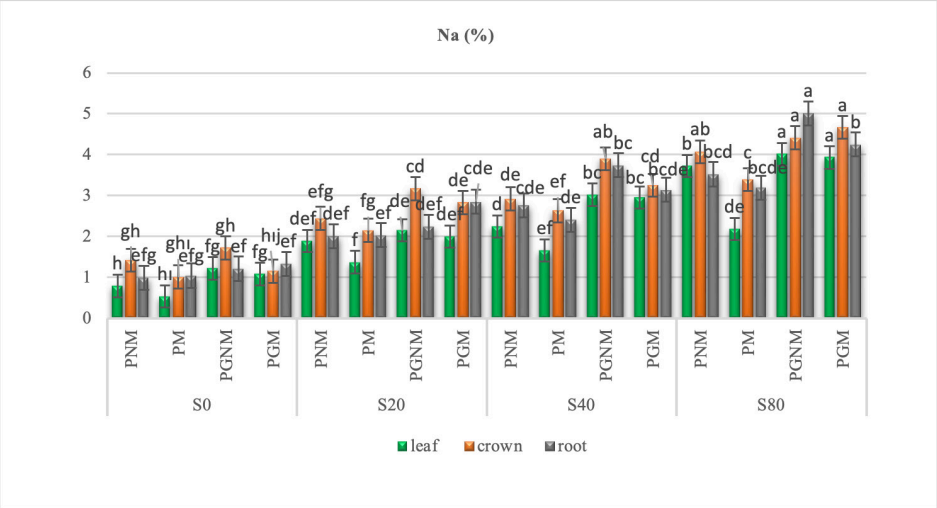

Figure 3. % Na contents of the treatments

CONCLUSION

Salinity is one of the most important factors that limit plant growth by negatively affecting soil fertility in agricultural production areas. Strawberries are among the plant species with the highest sensitivity to salt. In this study, it was determined that the application of mycorrhiza significantly affected the growth and development parameters of the Monterey strawberry variety under salt-stress conditions. It was concluded that the accumulation of Na^+ ions in the roots, crowns, and leaves should be considered in the resistance of strawberry plants under salty conditions. In the present study, it was observed that all values except the amount of Na decreased as salinity increased in all applications.

In conclusion, the use of mycorrhizal strains under stress conditions such as salinity had a significant effect on vegetative and generative growth in the Monterey strawberry variety. It was found that the use of mycorrhizal strains in strawberry cultivation significantly increased plant growth and development. Accordingly, it can be stated that the use of mycorrhizae to promote plant growth has more positive effects and is more beneficial to use in practice. In addition, it is important to determine the most suitable combinations by carrying out such studies on different varieties and species with different mycorrhizal strains, which will provide a source for field studies to be carried out in the future.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Emrah BAĞ: Investigation, Project administration, Writing, Funding acquisition; Beril KOCAMAN: Supervisor, Editing.

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RESEARCH ARTICLE

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Cytotoxic potential of selenium nanoparticles (SeNPs) derived from leaf extract of *Mentha longifolia* L.

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Abstract
The search for alternative methods in cancer treatments has been going on for many years. In the current study conducted for this purpose, selenium nanoparticles (ML-SeNPs) were produced from the aqueous leaf extract of Mentha longifolia L. easily and inexpensively, without harming the environment. The anticancer potential of ML-SeNPs on glioblastoma cell (U373), osteosarcoma cell (U2OS), and healthy retinal pigment epithelial cell (RPE-1) lines was determined by MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromid) test. For the test, ML-SeNPs were applied at 100, 300, and 600 µg/mL levels and interaction was provided for 24 and 48 hours. The survival rates (%) in RPE-1, U373, and U2OS cell lines in the 24-hour application were 107.49-98.89, 97.66-86.82, and 87.81-83.37, respectively. The viability rates (%) of the cells in 48 hours of application were 72.27-87.39, 68.17-73.48, and 81.00-84.67, respectively. In general, it was discovered that the cytotoxic effect of ML-SeNPs on RPE-1, U373, and U2OS cell lines was greater at low doses and increased over time. In-vivo studies that support the antiproliferative action of ML-SeNPs may boost the prospect of using them as therapeutic agents in potential cancer treatment procedures in the following years.
Keywords: Anticancer, *Mentha longifolia* L., SeNPs, U373, U2OS

INTRODUCTION

The increase in cancer cases worldwide in recent years is remarkable Who, (2022). According to the CBTRUS (The Central Brain Tumor Registry of the United States) Statistical Report, glioblastoma, the most common malignant central nervous system tumor, is the most aggressive form of brain cancer, with a 5-year overall survival rate of patients is 7% (Glioblastoma, 2022; Senhaji et al. 2022). Osteosarcoma is a very common form of malignant bone cancer, which is detected more in youth and young adults than in the previous cancer type, and the survival rate in patients is around 60-70% (Ni et al. 2022).

Conventional cancer treatment includes endocrinological therapy, surgical excision of cancerous tissues, and cell killing with chemotherapy or radiotherapy. Nevertheless, the patient's healthy cells, tissues, and organs are typically harmed by these treatments. (Ikram et al. 2021). In the recent several decades, efforts to produce medications with great therapeutic potency have intensified in order to decrease the aforementioned harm in patients. However, despite encouraging preclinical results for many novel medications, these results could not reflect the predicted effects in patients (Ntafoulis et al. 2023)During this time, with the advancement of nanotechnology, alternative nanomaterials to conventional pharmaceuticals emerged (Hatipoğlu et al. 2023; Baran et al. 2023;İpek et al.2023). Some of these nanomaterials are NP forms of elements such as silver,

gold, zinc, selenium, palladium and platinum synthesized from biological sources(Yang et al. 2022; Hatipoğlu et al. 2023; Ashrafet al. 2023; Hashem et al., 2022, Aktepe et al. 2021; Gholami-Shabani et al 2023).

Researchers are becoming more interested in SeNPs, which are nanomaterials with a wide range of applications, including electronics, biosensors, food packaging, medicine, optics, and catalysis (Hussain et al., 2023). One of the elements required by the human body is selenium. However, while low quantities of selenium enable adequate human body function, excessive doses (such as 3200 g and higher per day) are harmful (Srivastava et al. 2015). Selenium is essential for thyroid hormone metabolism and immunological function. Furthermore, selenium and SeNPs shield cells from free radical harm by enhancing the activity of antioxidant enzymes (glutathione peroxidase and thioredoxin reductase). Cancer, cardiovascular disease, and inflammatory illnesses have all been related to selenium insufficiency (Shoeibi et al. 2017; Pyrzynska et al. 2012).

SeNPs can be generated by physical, chemical and biological (green synthesis) approaches Aktepe et al. (2022). Physical and chemical procedures necessitate the use of costly and ecologically hazardous severe poisonous substances (Hatipoglu, 2021; Baran et al. 2021). As a result, as in other NPs, low-cost, environmentally friendly and green chemistry procedures that do not contain toxic chemicals have begun to be adopted in the production of SeNPs (Saranya et al., 2023; Baran et al., 2023). Furthermore, in the biosynthesis process of SeNPs, biomolecules such as polysaccharides, phenolic compounds, saponins, flavonoids, enzymes, tannins, amino acids, proteins, and sugars in plant extracts are evaluated as possible reducing and stabilizing factors Pyrzynska, (2021).

Mentha longifolia L., which belongs to the Lamiaceae family, has a square section, finely hairy and up to 1.5 m long, leaves up to 90 mm long and 22 mm wide, tiny flowers, rhizomatous, perennial, herbaceous, rapidly growing, medicinal and aromatic plant (Mohammad Hosein et al., 2017; Gharib et al. 2020; İpek et al., 2023 Patonay et al., 2021).Strong-smelling M. longifolia has spike-shaped inflorescences with numerous flowers (Bahadori et al., 2018). Puneh, punk, and wild mint are other names for this plant (Saeidi et al. 2014; Atalar et al. 2021). *M. longifolia* has 22 subspecies, indicating a high level of genetic variation. Because it is found in western and central Asia, temperate and subtropical Europe, and northern and southern Africa, it is regarded the world's most common wild mint taxon (Patonay et al., 2021). Its leaves or fresh shoots are usually used as a mint aroma and as a garnish in salads and prepared foods Mohammad Hosein et al. (2017). *M. longifolia* is used for the amelioration of cough, hypertension, cold, sinusitis, asthma, and digestive issues as a food additive and pharmaceutical ingredient Anwar et al., (2017). Because of their sedative, antimicrobial, antioxidant, antipruritic, anticancer, antispasmodic, antihistaminic, diuretic, anti-inflammatory, hepatoprotective, and biopesticide properties, *M. longifolia* plant extracts and/or essential oils have numerous applications in the pharmaceutical, food, and hygiene industries (Mokaberinejad et al., 2012; Ali et al. 2021). The primary synthetic method for producing selenium nanoparticles is chemical reduction, which makes use of a stabilizer and reducing agent. Stabilizer use, however, may impede the regular use of produced nanoparticles in biological applications, and because of its chemical makeup, stabilizer may also be hazardous. This work assesses the potential biological utility of selenium nanoparticles as an anticancer treatment and reports on a straightforward green production of the metal via phytochemical mediation.The purpose of this work was to reveal the anticancer activity of ML-SeNPs generated from the aqueous leaf extract of *M. longifolia* L. in an environmentally friendly way.

MATERIALS AND METHODS

Materials

M. longifolia L. used in the research was acquired from public bazaars in Diyarbakır (Türkiye). Sigma-Aldrich (USA) provided sodium selenite (Na_2SeO_3, 99% purity). The American Type Culture Collection (ATCC) cell lines U373 (glioblastoma), U2OS (osteosarcoma), and healthy RPE-1 (retinal pigment epithelial cell) were utilized to assess the cytotoxic effects of ML-SeNPs. MTT used in cytotoxic experiments was purchased from Merck (Germany). The cell lines were cultured in RPMI-1640 media (Sigma-Aldrich, USA).

Methods

Biosynthesis of plant compatible SeNPs

To synthesize SeNPs, a 1 M Na_2SeO_3 solution was produced. 75 mL of the extract was combined with 25 mL of Na_2SeO_3 solution and allowed to react at 30 °C for 4 hours. Following the observation of the color change, the resultant solution was centrifuged for 30 minutes (6000 rpm). The collected solid phase at the bottom was rinsed many times with distilled water. The prepared NPs were dried in an oven (80 °C / 48 hours). In a mortar, the solid portion was crushed. The NPs was saved for use in cytotoxic activity investigations.

Cytotoxic Activities of ML-SeNPs Via the MTT Assay

Selected cell lines were cultured in T75 flasks in RPMI-1640 media containing 10% FBS, 2 mM L-glutamine, and 100

units/mL penicillin/streptomycin and incubated at 37 °C in a 5% CO_2 atmosphere. When the cells had attained 80-90% confluence, they were removed from the flasks and their cell counts were quantified using the hemocytometric technique. Cells were injected in triplicate into 96-well plates in 90 L media for each well of U373, U2OS (5×10^3), and RPE-1 (10×10^3) cell lines (two on microplates to execute two distinct periods of 24 and 48 hours). The cells were allowed to cling to the microplate base for 24 hours.

The next day, different concentrations of ML-SeNPs (600, 300, and 100 g/mL) were added to the seeded plates. The cells in the control group were treated with ultrapure water. The MTT assay was utilized to detect changes in cell viability 24 and 48 hours after treatment. Each well containing cells received 10 L of the prepared MTT (5 mg/mL) solution and was incubated for 3 hours at 37 °C in a humid atmosphere containing 5% CO_2. The medium was withdrawn after 3 hours, and 100 L of DMSO was injected to each well. After 20 minutes in the shaker, the optical density (OD) values in the wells were measured using a UV-vis spectrophotometer (Multiskan GO, Thermofisher, USA) Irtegun and İpek (2023).

The absorbance values obtained by reading the control wells were averaged and regarded as 100% viable cell value. The absorbance readings from the ML-SeNP-treated wells were proportioned to the control absorbance value and accepted as % viability. MTT testing were carried out three times on various days. The inhibitor concentration value for ML-SeNPs was computed using the obtained data and the GraphPad Prism 8 tool. Cytotoxic experiments were conducted at Dicle University, Veterinary Faculty, Cell Culture Laboratory (Diyarbakır, Türkiye).

Statistical analysis

The SPSS package program (IBM, 21.0) was used to analyze the study's data. $P<0.05$ was used as the statistical significance level.

RESULTS AND DISCUSSION

The 24-hour MTT assay revealed that a 100 g/mL dosage of ML-SeNPs increased the survival of healthy RPE-1 cells while inhibiting malignant U373 and U2OS cells some (Figure 1 and Table 1).

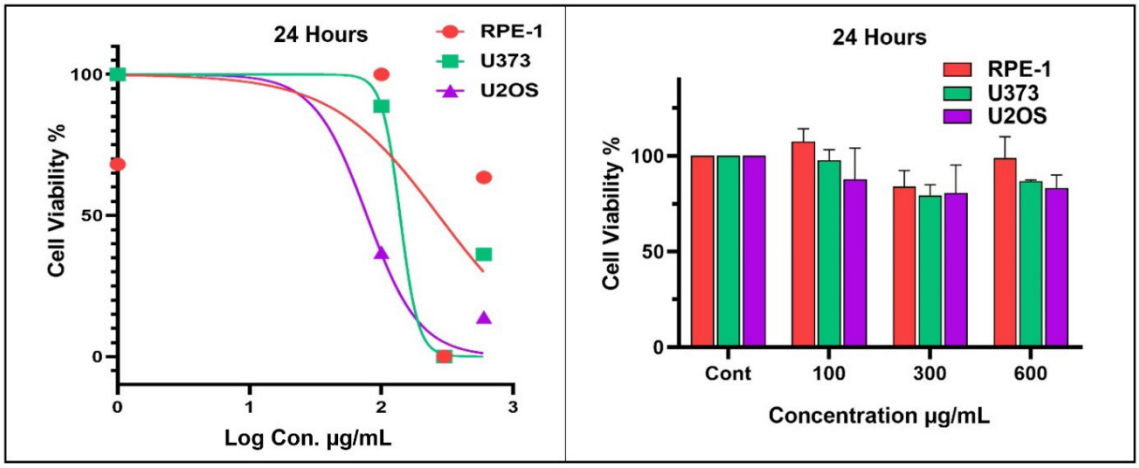

Figure 1. The half maximum inhibitory concentration (IC_{50}) results of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and healthy RPE-1 (retinal pigment epitelial cell) lines at 24 hours.

Increasing the dose of biogenic SeNPs to 300 µg/mL decreased the viability of all cell lines, whereas increasing this amount to 600 µg/mL increased the viability of all cell lines.

Table 1. Cytotoxic effects data of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and RPE-1 (retinal pigment epitelial cell) lines at 24 hours.

Cytotoxic effects ML-SeNPs on the cell lines (*n*=3, $\bar{X}\pm S\bar{x}$, *24* Hours)			
	100 µg/mL	**300 µg/mL**	**600 µg/mL**
RPE-1	107.49±06.62	83.97±08.36	98.89±11.16
U373	97.66±05.60	79.35±05.64	86.82±00.56
U2OS	87.81±16.24	80.66±14.55	83.37±06.59

The 48-hour MTT test revealed that a 100 g/mL dosage of ML-SeNPs produced a significant reduction in the viability of all cells (Figure 2 and Table 2). However, a progressive rise in cell viability was seen in line with the increase in NPs application dosage.

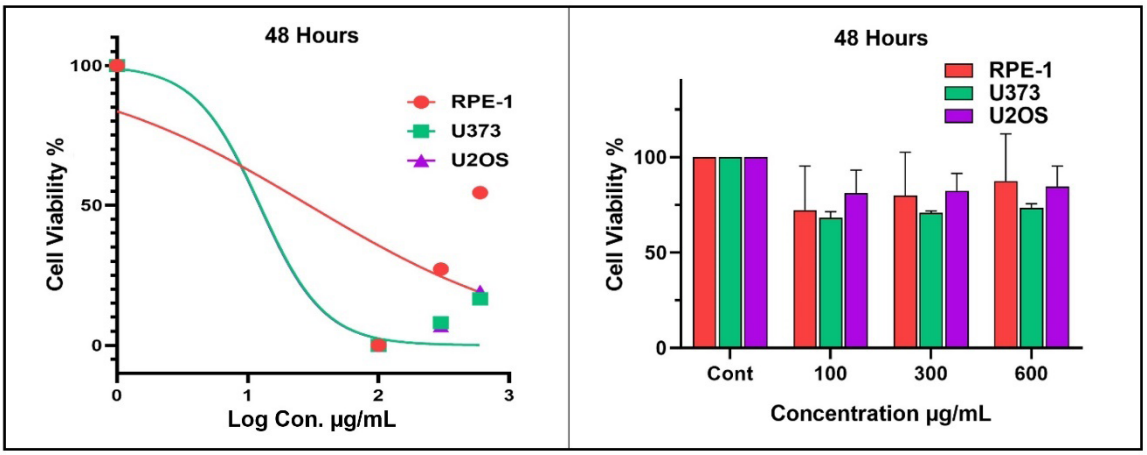

Figure 2. The half maximum inhibitory concentration (IC_{50}) results of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and healthy RPE-1 (retinal pigment epitelial cell) lines at 48 hours.

In this situation, the inhibitory impact of ML-SeNPs owing to dosage increase is restricted or negative in cells, whereas time is a factor that promotes inhibition in cells. SeNPs were shown to have high cytotoxic effects on T98-G (glioblastoma), Skov-3 (human ovarian adenocarcinoma), and 4T1 (mouse breast cancer cells) cells in three prior studies (Wadhwani et al., 2017:Gharbav et al., 2022).

Table 2. Cytotoxic effects data of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and RPE-1 (retinal pigment epithelial cell) lines at 48 hours.

Cytotoxic effects of ML-SeNPs on the cell lines (n=3, $\bar{X} \pm S\bar{x}$, *48* Hours)			
	100 µg/mL	**300 µg/mL**	**600 µg/mL**
RPE-1	72.27±22.98	79.82±22.67	87.39±24.81
U373	68.17±03.29	70.74±01.10	73.48±02.09
U2OS	81.00±12.12	82.33±09.07	84.67±10.60

Moreover, other studies found that SeNPs given at low dosages (5-100 g/mL) had a substantial inhibitory impact on HepG2 (hepatocellular carcinoma), MDA-MB-231 (breast cancer cells), and MCF-7 (breast cancer cells) (Cui et al., 2018; Cittrarasu et al., 2021).

One of the primary goals of nanomedicine is to solve the issues that are often associated with the use of conventional forms of drugs, particularly increased safety. Many studies highlight the unique medical applications of selenium nanoparticles (SeNP), which have various therapeutic benefits, including antioxidant, anti-inflammatory, anti-diabetic, and anti-tumor effects (Varlamova et al., 2021).Selenium nanoparticles (SeNPs) are considered superior to other metal nanoparticles, such as silver, gold, and platinum NPs, due to their better biocompatibility and in vivo degradability (Rajasekar & Kuppusamy, 2021).It is known that the pharmacological effect and toxicity of Se-based compounds depend on various factors, including concentration, redox, and type (Sonkusre & Cameotra, 2017; Varlamova et al., 2021). Several mechanisms have been suggested for selenium's anticancer activity, including cell cycle arrest, antioxidation, apoptosis, and interruption of the cell signaling pathway (Sonkusre & Cameotra, 2017).Nanoparticles had a different concentration-dependent effect on cancer cells of the studied human lines in our research. The study showed that SeNPs affected the survival of both cancer and normal cells in dose-dependent and time-dependent behaviors. The reduced cell viability was observed with increased time duration and decreased concentrations of SeNPs.

As a result, it is expected that by lowering the application dose, the nanomaterial employed in this study would have a stronger antiproliferative impact on cells.

CONCLUSION

The SeNPs used in this work were produced using a straightforward, economical, eco-friendly, and ecologically compatible phytofabrication process using leaf extract from *M. longifolia*. The produced SeNPs were nanoscale, naturally crystalline, negatively charged, spherical, and exceptionally stable.

The investigation was carried out to discover the effects of the produced nanomaterial on cancer cells and to manufacture the plant-based NP form of selenium, which is a vital mineral for human health. When the study's findings were analyzed, it was discovered that modest dosages of ML-SeNPs had a greater antiproliferative impact on cancer cells, and that waiting time amplified this effect to some extent. In order to more clearly indicate the anticancer potential of ML-SeNPs, future investigations should minimize the nanomaterial application dosage. Better yet, if in-vivo research supports the lethal ability of ML-SeNPs, this nanomaterial may be a stronger option for involvement in prospective cancer therapy procedures.

COMPLIANCE WITH ETCICAL STANDARTS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors in this study have no conflicts of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

This study does not require an ethics committee.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Antagonistic activities of mycoparasitic *Pythium* species against *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea* on tomatoes

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Abstract
In this study, antagonistic effects of *Pythium acanthophoron*, *P. lycopersicum*, *P. oligandrum* and *P. paroecandrum* against *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea* were investigated by *in vitro* and *in vivo* trials. *In vitro* mycoparasitic activities of *Pythium* species were determined by dual culture, inverted plate culture and agar diffusion tests. As a result of dual culture tests, suppressive effects of all mycoparasites were over 70% against mycelial growth of the pathogens. Inverted plate tests showed that antagonistic effects of mycoparasites regarding their volatile compounds were rather low. In the agar diffusion test, all mycoparasites showed antibiosis effect, however *P. lycopersicum* had the highest suppressive effect on both pathogens. In pot trials, mycoparasites were effective to protect tomato seedlings when pathogens were seperately inoculated, and suppressed the symptoms. When two pathogens were inoculated together, *P. paroecandrum* was ineffective against *B. cinerea*, but decreased the severity of wilt symptoms, while other mycoparasites totally inhibited both diseases. Chromatographic analyses made by using leaf samples taken 12, 24, 48 and 72 hours after pathogen inoculation showed meaningful increase on chlorogenic acid, caffeic acid and epicatechine, in the samples taken 48 hours after inoculation. Analyses after the inoculations of tomatoes with the mycoparasites and/or pathogens showed that mycoparasites also caused increase in the amounts of phenolics. This indicated that the mycoparasites could be effective to induce defense mechanisms of tomato plants against pathogens. Among them, *P. oligandrum* can be mentioned as the most effective mycoparasite regarding the induction of phenolics.
Keywords: *Solanum lycopersicum*, Fusarium wilt, Gray mold, Biocontrol, *Pythium* spp.

INTRODUCTION

Root, stem and fruit rots, damping off and wilt diseases caused by soil-borne plant pathogens are among the factors limiting the yield and crop quality. *Fusarium oxysporum* and *Botrytis cinerea* are widespread and important pathogens causing diseases on various crops (Lamichhane et al. 2017). *F. oxysporum* is one of the most common pathogens which has pathogenic forms specialized to 120-150 different host plants (Attitalla et al. 2004; Bogale et al. 2007; Kinyoda et al. 2022). Each form has one or more vegetative compatibility groups and one form generally causes disease on one host plant. There are two known special forms, *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (Sacc.) W. C. Snyder & H. N. Hans and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) (Jarvis & Shoemaker) causing wilt and, crown and root rot diseases on tomatoes, respectively (Boix-Ruíz et al. 2015). *Botrytis cinerea* Fr., another important fungal agent of tomato, causing gray mold disease, is also a very common and polyphagous pathogen, which is known to

have more than 200 hosts (Panno et al. 2021). It can infect leaves, flowers, stem and fruits of tomatoes and damage all plant in a very short time (Dik and Wubben, 2004). Control of both pathogens is difficult, since they can form resistant structures and survive in soil for many years. Mainly chemical control is used besides cultural methods like crop rotation and use of resistant varieties. However, because of the well-known negative side effects of fungicides on environment and human health (Panth et al. 2020), biological control, which is known to be a safer method, gained importance and various studies were made on the use of different fungal and bacterial agents against plant pathogens (Heydari and Pessarakli 2010; Pandit et al. 2022).

Mycoparasitic *Pythium* species are among the biological agents used against different plant pathogens (Paulitz and Baker, 1987; Martin and Hancock, 1987; Lodha and Webster, 1990; Ağaner et al. 2021). *Pythium oligandrum* Drechsler (Deacon, 1976), *P. acanthicum* Drechsler (Deacon and Henry, 1978), *P. nunn* Lifshitz, Stanghellini & Baker (Lifshitz et al. 1984), *P. periplocum* Drechsler (Hockenhull et al. 1992), *P. acanthophoron* Sideris and *P. mycoparasiticum* Deacon, S.A.K. Laing & L.A. Berry (Jones and Deacon, 1995) were the first *Pythium* species determined to have mycoparasitic activity. *P. radiosum* Paul (Paul, 1999), *P. contiguanum* Paul (Paul, 2000), *P. canariense* Paul (Paul, 2002), *P. bifurcatum* Paul (Paul, 2003), *P. paroecandrum* Drechsler (Abdelghani et al. 2004), *P. citrinum* Paul (Paul, 2006), *P. lycopersicum* Karaca, Tepedelen Belgouthi and Paul (Karaca et al. 2008), and *P. amasculinum* Yu (Tepedelen, 2008) were subsequently described as mycoparasites. *P. oligandrum* is the most common and known mycoparasite and detailed studies were made on its mycoparasitic activity against different plant pathogens on various crops (Al-Hamadani and Cooke, 1983; Abdelzaher et al. 1997; Benhamou et al. 1997; Al-Rahawi and Hancock, 1998; Madsen and Neegaard, 1999; Picard et al. 2000a; Takenaka et al. 2003; Bělonožníková et al. 2022). However, there is less work on antagonism mechanisms of other *Pythium* species. The aim of this research is to determine *in vitro* and *in vivo* antagonistic activities of *P. acanthophoron*, *P. lycopersicum*, *P. oligandrum* and *P. paroecandrum,* isolated from Mediterranean region of Türkiye, against two important tomato pathogens; F. *oxysporum* f. sp. *lycopersici* and *B. cinerea*.

MATERIALS AND METHODS

Determination of the *in vitro* antagonistic activities of the mycoparasitic *Pythium* species

In the study, *F. oxysporum* f. sp. *lycopersici* (FOL) isolate obtained from Western Mediterranean Agricultural Research Institute (Antalya, Türkiye) and *B. cinerea* isolated from diseased tomato plants taken from greenhouses in Antalya were used as pathogens and *P. lycopersicum* (AD7-6), *P. acanthophoron* (IS7-2), *P. pareocandrum* (MKB2-1) and *P. oligandrum* (AK4T-1) isolates obtained from soil samples of different crops in the Mediterranean region of Türkiye, were used as mycoparasites. *B. cinerea* was isolated from the stem sections of tomato plants showing gray mold symptoms. Stem pieces including healthy and diseased tissue were surface sterilized with 1% NaOCl solution, blotted dry and transferred to Potato Dextrose Agar (PDA) plates. Growing hyphal tips were transferred to obtain pure cultures after incubation at 21°C for a few days. Mycoparasites were isolated from soil by using surface soil dilution plate (SSDP) method (Tepedelen, 2008).

In vitro antagonistic activities of mycoparasitic *Pythium* species were investigated by dual culture, inverted plates and agar diffusion methods. To determine the parasitic activities of *Pythium* species against pathogens, dual culture plate method was used. Mycelial disks of each pathogen and mycoparasite, taken from the growing edge of agar plates, were transferred to opposite sides of 9 cm diameter Petri plates with PDA and incubated at 25°C for 4 days. Plates inoculated only with pathogens served as controls and 3 replicate plates were used for each application. Radial growth of pathogen colonies were compared with controls and rate of inhibition (%) was calculated (Elshahawy and El-Mohamedy, 2019).

Effects of the possible volatile metabolites of the mycoparasites were investigated by inverted plate culture technique. Seperate PDA plates were inoculated with a single agar plug of pathogens and mycoparasites. After removing the lids, plates with mycoparasites were inverted over plates with pathogens and covered by parafilm. Similar plates without mycoparasites served as controls. After 4 days incubation at 25°C, growth inhibition was calculated (Pavitra et al. 2022).

Agar diffusion test was used to determine the possible antibiotic production of the mycoparasites. Spore suspensions of the pathogenic fungi (10^6 spores/ml) were spread over the plates with PDA. Sterilized culture filtrates (100 µl) of the mycoparasites grown in liquid medium were aseptically pipetted to 5 mm diameter wells made by a sterile cork borer. Plates were incubated at 25°C for 4 days and diameter of the inhibition zones around the wells were measured (Karunasinghe et al. 2020).

Determination of the *in vivo* biocontrol activities of the mycoparasitic *Pythium* species

At first, virulence of the mycoparasitic *Pythium* isolates were investigated and compared by the pathogenic species *P. deliense,* isolated from diseased tomato seedlings. Sterile sand, water and cornmeal mixture in 9:2:1 (v:v:v) rates were

used to obtain *Pythium* inoculum. Four weeks old tomato seedlings (cv. Caroca F1) were used in the pathogenicity tests. Tomato seedlings were transferred to the pots containing sterile soil mixture with *Pythium* inoculum (>200 propagules/g). Inoculum concentrations were checked by SSDP method given above. Pots without inoculum were used as controls and three replicate pots each with one seedling were used for each application. Virulence of the isolates was evaluated when severe symptoms on the *P. deliense* inoculated seedlings were observed. Disease severity was determined by using 0-3 scale modified from Botha et al. (1992), where; 0=no symptom, 1=small lesions on the roots or crown, slight wilting, 2=larger lesions on the stem, moderate wilting, 3=lesions girdling the stem, severe wilting or dead seedling.

Regarding the investigation of the interactions among mycoparasites and pathogens, tomato plants (cv. Caroca F1) were inoculated with pathogens with or without mycoparasites and plant growth and disease symptoms were evaluated. Mycoparasitic *Pythium* inoculum was incorporated into soil mixture as mentioned above. Roots of the tomato seedlings were wounded by cutting and inoculated by dipping in FOL spore suspension with a concentration of $2x10^7$ spores/ml for 30 minutes. *B. cinerea* inoculation was performed by spraying tomato leaves with the spore suspension of the pathogen in same concentration. Three replicate pots were used and pots without mycoparasite inoculum were used as controls. Plants were incubated in a climatic room with 22˚C temperature, 12h: 12h light and dark conditions and watered when necessary. After two weeks incubation, plants were observed for disease symptoms and evaluated by using special scales for each disease. For FOL, 0-3 scale modified from Ozbay and Steven (2004) where; 0=no visible symptom, 1=slight color change on vascular bundles, 2=severe color change on vascular bundles, slight wilting of leaves, 3=severe wilting, dried or dead plant, was used. Similarly, 0-3 scale modified from Lou et al. (2011) where; 0= no visible symptom, 1=less than 25% of leaf area was affected, 2=less than 50% of leaf area was affected, 3=more than 50% of leaf area was affected, was used for *B. cinerea*.

For the determination of the effects of mycoparasites on the resistance mechanisms of tomato plants, amounts of the phenolic compounds in tomato leaves; 12, 24, 48 and 72 hours after mycoparasite and/or pathogen inoculation, were determined by chromatographic analyses. High performance liquid chromatography (HPLC) method was used to detect and quantify the phenolic compounds of the leaf samples taken from the tomato plants inoculated with pathogens and/or mycoparasites. Two tomato cultivars, one of them known as relatively susceptible (cv. H-2274) and the other more tolerant (cv. Newton) to the diseases, were used in the experiment. Analyses were performed in the YETEM-Innovative Technologies Application and Research Center of Süleyman Demirel University, by using Shimadzu model HPLC (Shimadzu Corp., Kyoto, Japan) with a SCL-10A vp system controller, a LC-10AD vp pump, Diode Array Detector with wavelength at 278 nm, a SIL10AD vp autosampler, CTO-10 A vp column heater and a DGU-14a degasser and Agilent Eclipse Zorbax C18 column. Standarts of 12 phenolic compounds (gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, syringic acid, *p*-coumaric acid, ferulic acid, cinapic acid, *o*-coumaric acid, cinnamic acide and quercetin) were obtained from Sigma (St. Louis, MO, USA).

All trials were repeated two times and SPSS 23® program was used for statistical anayses. Data were subjected to analyses of variance and means were compared by Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

In vitro antagonistic activities of the *Pythium* species

Mycelial growth of *B. cinerea* and FOL was inhibited in different rates by the four antagonistic *Pythium* species used in the study. It was found that the parasitic activities of the antagonists were higher than their antibiosis related effects (Table 1, Figure 1). In the dual plate culture method, all antagonists used in the study clearly inhibited the mycelial growth of both pathogens. The highest rates of inhibition for both pathogens were obtained by *P. lycopersicum*. *P. acanthophoron* showed mean inhibition rate of 80.74% on the mycelial growth of *B. cinerea* and was statistically arranged in the same group with *P. lycopersicum*. *P. oligandrum* and *P. paroecandrum* formed the second statistical group with growth inhibitions over 70%. Similarly, high rates of mycelial growth inhibition were observed for FOL with all mycoparasites. *P. lycopersicum* inhibited the mycelial growth of the pathogen about 83%, whereas other three mycoparasites showed inhibitions over 70%. These findings are coherent with those of El-Katatny et al. (2005) and Attia et al. (2022), who reported that *P. oligandrum* completely overgrew *F. oxysporum* and *P. ultimum* var. *ultimum* in dual cultures. It was determined in the inverted plate method that the inhibitory effects of *Pythium* species related with their volatile components were rather low. Highest inhibitory effect against *B. cinerea* was obtained with *P. oligandrum*, while *P. lycopersicum* showed the highest effect against FOL. *P. acanthophoron* yielded the minimum inhibition rates against both pathogens. Despite our findings, El-Katatny et al. (2005) reported that *P. oligandrum* almost totally inhibited the growth of *P. ultimum* and *F. oxysporum* with its volatile metabolites. Agar diffusion test showed that all four mycoparasitic species produced metabolites with antibiotic activity in varying degrees. Culture filtrate of *P. lycopersicum* caused the widest inhibition zones on the mycelial growth of *B. cinerea* and FOL, while the narrowest zones were obtained by *P. oligandrum* for both pathogens. Previously, *P. oligandrum* was found to have no

antibiotic production as a result of a similar test using two plant pathogenic fungi (El-Katatny et al. 2005). This may because of the differing activities of the isolates or the susceptibility of host organisms.

Table 1. Inhibition of *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) mycelial growth caused by the mycoparasitic *Pythium* species in dual plate, inverted plate and agar diffusion tests.

Mycoparasitic *Pythium* species	Mean inhibition rates (%)				Inhibition zone diameter (mm)	
	Dual plate culture		Inverted plate test		Agar diffusion test	
	B. cinerea	FOL	B. cinerea	FOL	B. cinerea	FOL
P. acanthophoron	80.74 a[x]	74.81 b	6.32 c	5.99 c	19.00 b	17.00 bc
P. lycopersicum	84.44 a	82.59 a	6.90 b	7.55 a	25.00 a	21.00 a
P. oligandrum	73.33 b	74.81 b	7.30 a	6.36 bc	17.00 b	15.00 c
P. paroecandrum	78.88 b	72.96 b	6.97 b	7.43 b	23.00 a	19.00 ab

[x]Values in the same column shown by the same letter were statistically not different from each other according to Tukey's test (P≤0.05).

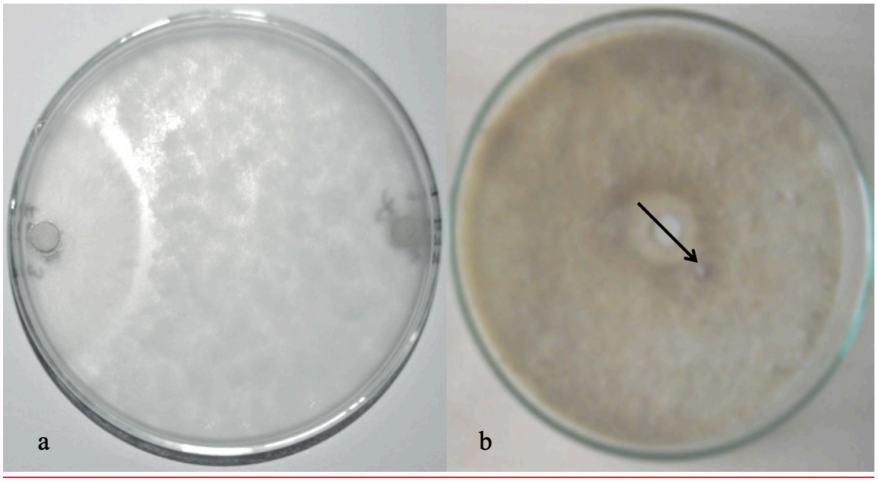

Figure 1. Inhibition of the *in vitro* mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* a) by *P. lycopersicum* (on the right) in dual plate culture and b) by *P. acanthophoron* in agar diffusion test (arrow shows the inhibition zone).

In our study, it was determined that the inhibitory effects of *Pythium* species showed slight variations and could be changed depending on the pathogens. Similarly, Jones and Deacon (1995) reported that the mycoparasitic performance of *P. acanthophoron* was better than *P. mycoparasiticum*, but lower than *P. oligandrum*. Varying degrees of mycoparasitic activities also mentioned for *P. acanthicum, P. oligandrum* and *P. periplocum* against pathogens under different *in vitro* conditions (Ali-Shtayeh and Saleh, 1999). Thus, it can be mentioned that the mycoparasitic performances of *Pythium* species could be changed according to the isolates, species, host pathogens and conditions during mycoparasitism.

Virulence of the mycoparasitic *Pythium* species on tomato seedlings

In the pathogenicity test, *P. oligandrum* caused no symptoms, while *P. deliense*, used as pathogen control, caused severe root rot and depressed the growth of tomato plants. *P. acantophoron*, *P. lycopersicum* and *P. pareocandrum* caused slight color change on the roots, but they did not cause any disease symptoms on the upper ground parts of the plants (Figure 2).

P. oligandrum was previously isolated from a number of plants in different countries but its virulence was known to be rather low and this species is known as an effective mycoparasite (Plaats-Niterink, 1981; Yu and Ma, 1989). *P. acanthophoron* was originally isolated from *Ananas sativus* and was mentioned as a weak pathogen on this plant by Sideris (Plaats-Niterink, 1981). Later, it was reported to be a mycoparasite (Lodha and Webster, 1990; Jones and Deacon, 1995). Pepper, bean and pine plants were also reported as other hosts of this species (Hall, 1998). *P. paroecandrum* was isolated from various plants and reported to be weakly pathogenic (Plaats-Niterink, 1981). In a study made in Türkiye, it was reported that *P. paroecandrum* caused slight lesions on corn, wheat, bean and cucumber plants, moderate symptoms on tomato and sunflower plants and caused severe post-emergence damping-off symptoms on pepper, tobacco and sugarbeet plants (Hatat, 1995). *P. acanthophoron, P. lycopersicum* and *P. paroecandrum* caused slight decrease in seedling emergence and moderate browning on the roots of tobacco seedlings (Karabuğa, 2011).

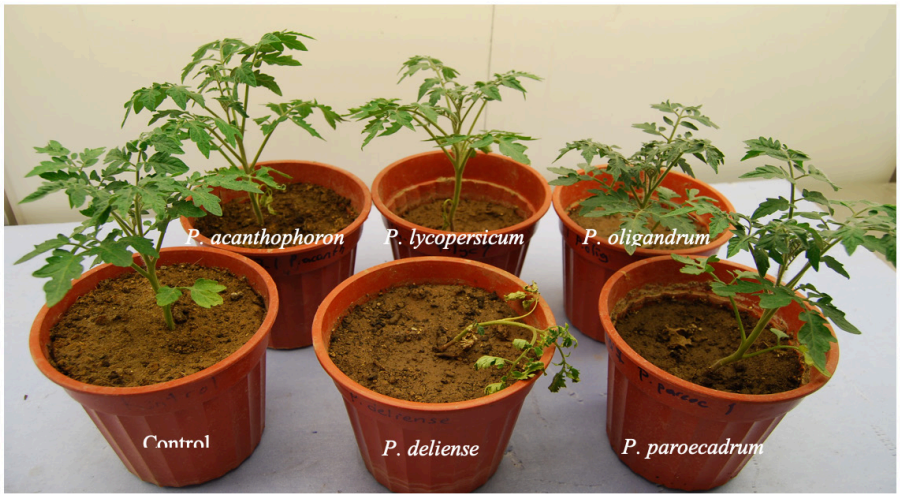

Figure 2. Effects of *Pythium* species on growth of tomato plants.

Biocontrol activities of the mycoparasitic *Pythium* species against two pathogens on tomato plants

In the trials performed to determine the preventive effects of mycoparasitic *Pythium* species against Fusarium wilt and gray mold diseases of tomato, all mycoparasites suppressed FOL and prevented tomato plants from wilting when inoculated with the pathogen, while only FOL inoculated plants showed severe wilting symptoms and dried (Figure 3). Plants inoculated with *B. cinerea* alone showed severe symptoms on the leaves, while those inoculated both with the mycoparasites and the pathogen showed slight symptoms and statistically arranged in the same group with control, except plants inoculated with *P. paroecandrum* which showed slight symptoms. Similarly, on the plants inoculated with both pathogens, again all mycoparasites except *P. paroecandrum* suppressed both pathogens and slight symptoms of both pathogens were observed only on plants inoculated with pathogens and *P. paroecandrum* (Table 2). This showed that *P. pareocandrum* isolate could suppress but not totally inhibit pathogen infections on tomato plants.

Pythium species can be effective as biological control agents by different mechanisms. Interactions between *P. oligandrum* and *Phytophthora parasitica* cells was observed by electron microscopy and it was determined that mycoparasitism was based on enzyme activity differing from other mycoparasite-pathogen interactions (Picard et al. 2000b). Another study revealed that *P. oligandrum* can affect pathogens by the synthesis of chitinase and β-1,3-glucanase enzymes (El-Katatny et al. 2005).

Figure 3. Preventive effect of *Pythium oligandrum* against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) infection on tomato plants

Table 2. Mean scale values and mean disease severity rates (%) of tomato plants inoculated with the pathogens and/or mycoparasitic *Pythium* species.

Genotypes	Fusarium oxysporum f. sp. lycopersici (FOL)		Botrytis cinerea (BC)	
	Mean scale value	Mean disease severity (%)	Mean scale value	Mean disease severity (%)
Control	0.00[x]b[y]	0.00	0.00[z]c	0.00
FOL	3.00 a	100.00	-	-
BC	-	-	3.00 a	100.00
P. acanthophoron+FOL	0.00 b	0.00	-	-
P. acanthophoron+BC	-	-	0.00 c	0.00
P. acanthophoron+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. lycopersicum+FOL	0.00 b	0.00	-	-
P. lycopersicum+BC	-	-	0.33 bc	11.11
P. lycopersicum+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. oligandrum+FOL	0.00 b	0.00	-	-
P. oligandrum+BC	-	-	0.33 bc	11.11
P. oligandrum+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. paroecandrum+FOL	1.00 ab	33.33	-	-
P. paroecandrum+BC	-	-	1.00 abc	33.33
P. paroecandrum+FOL+BC	1.00 ab	33.33	2.00 ab	66.67

[x] 0-3 scale for FOL; 0=no visible symptom, 1=slight color change on vascular bundles, 2=severe color change on vascular bundles, slight wilting of leaves, 3=severe wilting, dried or dead plant was used.
[y] Means on the same column shown by same letters were not significantly different from each other according to Tukey's test ($p \leq 0.05$)
[z] 0-3 scale for *B. cinerea*; 0= no visible symptom, 1=less than 25% of leaf area was affected, 2=less than 50% of leaf area was affected, 3=more than 50% of leaf area was affected.

Effects of mycoparasitic *Pythium* species and pathogens on phenolic contents of tomato plants

In the preliminary analyses made by using tomato leaf samples taken 12, 24, 48 and 72 hours after inoculation with FOL, 8 of the 12 standard phenolic compounds; gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, syringic acid, *p*-coumaric acid and *o*-coumaric acid were detected. It was found that chlorogenic acid, caffeic acid and epicatechin amounts of the leaves showed the most meaningful increases, 48 hours after inoculation. Thus, amounts of caffeic acid, chlorogenic acid and epicatechin of the leaf samples of two tomato cultivars, inoculated with the mycoparasites and pathogens, were evaluated and compared, 48 hours after the inoculations. As a result of the chromatographic analysis, it was found that the inoculations of some of the mycoparasites caused increase on the amounts of phenolics when compared to control plants. However, increase of epicatechin amounts were higher in the leaf samples taken from the resistant cultivar Newton. In addition, inoculations of the both pathogens with mycoparasites caused more prominent increases in the amounts of phenolics, on the same cultivar. Among the mycoparasitic *Pythium* species, *P. oligandrum* inoculated with the both pathogens yielded the highest phenolic amounts (Table 3).

Our results are consistent with the previous similar studies. Pharand et al. (2002) found that *P. oligandrum* inoculation decreased wilt symptoms on tomato plants by inducing the defense mechanism of the plants against *F. oxysporum* f. sp. *lycopersici*. In another study, it was determined that *P. oligandrum* colonization on tomato roots prevented root rot caused by *Pythium* species and also effective against *B. cinerea* by means of the induction of plant defense (Le Floch et al. 2003). Biochemical analyses made after *P. oligandrum* inoculation of tomato plants showed that amounts of phenolics increased after 3 hours and rishitin synthesis started at 14th hour. Some responses of the host plant showed regular increases during 34 hours (Le Floch et al. 2005).

Table 3. Mean amounts of caffeic acid, chlorogenic acid and epicatechin in the leaf samples of susceptible (cv. H-2274) and tolerant (cv. Newton) tomato plants 48 hours after inoculations of mycoparasites and/or pathogens.

Applications	Mean amounts of phenolics (µg/g)					
	Caffeic acid		Chlorogenic acid		Epicatechin	
	H-2274	Newton	H-2274	Newton	H-2274	Newton
Control	1.9 gh[x]	1.1 h	4.1 cdef	6.8 e	4.6 f	1.7 h
P. acanthophoron	1.3 h	5.3 def	2.5 ef	7.8 de	4.8 ef	18.6 de
P. lycopersicum	1.7 gh	3.9 def	3.4 ef	5.8 e	4.4 f	14.4 ef
P. oligandrum	2.3 gh	5.6 de	4.4 cde	6.6 e	5.4 ef	14.1 ef
P. paroecandrum	2.4 fgh	5.4 def	4.4 cde	8.8 de	8.7 def	17.9 e
BC	1.9 gh	3.0 efgh	4.2 cdef	8.5 de	4.7 ef	3.0 gh
P. acanthophoron+BC	1.5 gh	4.0 def	2.4 f	15.0 bc	5.1 f	1.4 h
P. lycopersicum+BC	1.7 gh	3.0 efgh	2.8 def	17.9 ab	4.6 ef	2.2 h
P. oligandrum+BC	2.4 fgh	5.3 def	4.4 cde	18.6 ab	5.9 ef	3.0 gh
P. paroecandrum+BC	2.5 efgh	3.2 defgh	4.5 bcd	16.2 abc	8.9 def	UD h
FOL	3.6 cd	1.2 gh	6.0 ab	5.3 e	9.6 de	1.7 h
P. acanthophoron+FOL	4.0 bc	3.4 fgh	5.5 abc	12.3 cd	12.9 cd	2.0 h
P. lycopersicum+FOL	4.2 bc	5.5 d	5.2 abcd	19.1 a	17.2 c	2.8 h
P. oligandrum+FOL	3.6 cd	1.1 h	4.6 bcd	7.1 e	17.0 c	UD h
P. paroecandrum+FOL	4.2 ab	3.8 defg	5.4 a	9.0 de	17.7 f	8.4 fg
P. acanthophoron+FOL+BC	2.3 gh	3.9 def	4.3 abcd	UD[y] f	5.7 ef	16.8 e
P. lycopersicum+FOL+BC	2.7 defg	11.1 c	3.9 cdef	14.1 bc	16.3 c	27.0 cd
P. oligandrum+FOL+BC	5.9 a	13.7 b	7.9 a	14.9 bc	28.6 a	36.0 b
P. paroecandrum+FOL+BC	3.4 cdef	18.1 a	4.2 cdef	20.8 a	14.2 c	59.1 a

[x] Means on the same column shown by same letters were not significantly different from each other according to Tukey's test ($p \leq 0.05$).

[y] Under detectable level.

CONCLUSION

All the mycoparasitic *Pythium* species used in this study did not cause serious disease symptom on tomato plants. However, they prevent the plants from diseases when they inoculated together with pathogens. In addition to their suppressive effect on diseases, mycoparasites also induced plant defense. As a result of this study, it was shown that not only *P. oligandrum*, but also the other mycoparasitic *Pythium* species can be effective to suppress plant diseases with different antagonistic mechanisms and can be used as biological control agents against some soil-borne plant pathogens. However, detailed studies should be made to investigate their efficiency under field conditions with different host-pathogen combinations.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that they have no conflicts of interest.

Author contribution

Both authors designed the research study, MA performed the research under the supervision of GK. The contribution of the authors on the preparation of the manuscript is equal. The authors read and approved the final manuscript and verify that the text, figures and tables are original.

Ethical approval

Ethics committee approval is not required since the article does not contain studies with human or animals.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Detection of artichoke on seedling based on YOLOV5 model

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Abstract

Robotic systems have become essential in the industrial field today. Robotic systems used in many areas of industry enable the development of mechanization of agriculture. Researches in recent years have focused on the introduction of automatic systems and robot prototypes in the field of agriculture in order to reduce production costs. The developed smart harvest robots are systems that can work uninterrupted for hours and guarantee minimum cost and high production. The main element of these systems is the determination of the location of the product to be harvested by image processing. In addition to the programs used for image processing, deep learning models have become popular today. Deep learning techniques offer high accuracy in analyzing and processing agricultural data. Due to this feature, the use of deep learning techniques in agriculture is becoming increasingly widespread. During the harvest of the artichoke, its head should generally be cut off with one or two leaves. One main head and usually two side heads occur from one shoot. Harvest maturity degree is the time when the heads reach 2/3 of their size, depending on the variety character. In this study, classification was made by using the deep learning method, considering the head size of the fruit. YOLOv5 (nano-small-medium and large models) was used for the deep learning method. All metric values of the models were examined. It was observed that the most successful model was the model trained with the YOLOv5n algorithm, 640x640 sized images with 20 Batch, 90 Epoch. Model values results were examined as "metrics/precision", "metrics/recall", "metrics/mAP_0.5" and "metrics/mAP_0.5:0.95". These are key metrics that measure the detection success of a model and indicate the performance of the relevant model on the validation dataset. It was determined that the metric data of the "YOLOv5 nano" model was higher compared to other models. The measured value was Model 1= Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n. Hence, it was understood that "Model 1" was the best detection model to be used in separating artichokes from branches in robotic artichoke harvesting.

Keywords: Deep learning,YOLOv5, Description,Classification

INTRODUCTION

Deep learning is an important topic in the field of machine learning and is used in many application areas. This technique is used to identify and learn complex patterns from large data sets. Deep learning algorithms consist of neural networks, and these networks are arranged in layers. Each layer takes the outputs of the previous layer as input and learns more complex features and patterns using these outputs. In this way, deep learning algorithms can discover hidden

structures in data and perform complex tasks. Deep learning is a subfield of machine learning that focuses on training artificial neural networks with multiple layers to learn and extract high-level representations from complex data (Deng, 2014). Deep learning is used in many different application areas. Examples of usage areas include image processing, deep learning algorithms, object detection and recognition. In addition, deep learning techniques are used in the field of natural language processing and successful results are achieved in tasks such as text classification, language translation and speech recognition. By analyzing large amounts of data, deep learning algorithms can identify patterns and relationships and use this information to make predictions based on new data. Due to these characteristics, deep learning forms the basis of artificial intelligence applications used in medicine, finance, automotive and many other industries. Deep learning algorithms consist of neural networks, and these networks are organized in layers. In deep learning, neural networks consist of multiple layers of interconnected nodes, known as neurons, that process and transform input data. Each layer in the network receives input from the previous layer and transfers its output to the next layer, enabling hierarchical extraction of features and patterns. While layers closer to the input are responsible for learning low-level features, deeper layers learn more abstract and complex representations (Deng, 2014).

One of the most important advantages of deep learning is that it can automatically learn features from raw data, eliminating the need for manual feature engineering. Deep learning models can learn hierarchical representations directly from data, and this enables them to capture complex patterns and relationships that may be difficult to define clearly. This makes deep learning especially effective in processing large and unstructured data sets such as images, text and audio (Akkus et al., 2017). Deep learning has begun to be widely used in many fields, from medicine to agriculture. It has enabled increasing efficiency, productivity and sustainability in agricultural practices. Various studies on this subject have investigated the application of deep learning in agriculture and highlighted its benefits and challenges. One of the promising areas of deep learning is the development of highly autonomous machines for agriculture. These machines can push safety standards and improve the overall efficiency of agricultural operations (Kamilaris et al., 2018). Another application of deep learning in agriculture is in the field of smart agriculture. Deep learning algorithms can be used to analyze large amounts of data collected from sensors, drones, and satellites, and allow farmers to take data-based decisions regarding crop management, irrigation, and pest control (Yang et al., 2022). This can lead to increased crop yields, reduced resource use and increased sustainability. Deep learning can also be used for plant stress monitoring, crop load prediction, and harvesting in agriculture (Gao et al., 2020). Deep learning algorithms can detect and classify plant diseases by analyzing images and data collected from various sources, and enable early intervention and prevention of crop losses (Saleem et al., 2019). This can significantly increase the productivity and efficiency of disease management in agriculture. In addition to crop management, deep learning methods can also be applied to other aspects of agriculture, such as anomaly detection in Internet of Things (IoT) time series data (Cheng et al., 2022). Precision agriculture is another area where deep learning can produce a significant impact. Deep learning can increase the efficiency and sustainability of precision agriculture by optimizing the control of agricultural production systems, managing agricultural economic systems, and processing agricultural information (Alreshidi, 2019). Despite the numerous benefits of deep learning in agriculture, there are also challenges that need to be discussed. These include the need for a large amount of labeled training data, the interpretability of deep learning models, and the integration of deep learning algorithms into existing agricultural systems (Ryo et al., 2022). Another study by Yang et al. (2022) focused on a small number of learnings in smart agriculture. In the study, the developments, applications and challenges of few-shot learning, which was a subfield of deep learning that aimed to train models with limited labeled data, were discussed. The study highlighted the potential of few-shot learning in discussing the problem of data scarcity in agriculture and enabling the development of accurate and efficient models for a variety of tasks. In the study conducted by Cheng et al. (2022), the use of generative adversarial networks (GANs) with attention mechanisms for anomaly detection in smart agriculture was investigated. In the study, a new approach that combined GANs and attention mechanisms to detect anomalies in time series data collected from IoT devices in agricultural systems, was proposed. Besides, Xiao et al. (2022) presented a deep learning-based method for the detection of weeds in vegetables. In the study, a new approach that used deep learning algorithms to accurately identify and classify weeds in vegetable crops, was proposed. As a result of the study, they identified the potential of deep learning in weed control, which was a critical task in agriculture to provide optimum growth and yield of crops.

Overall, deep learning shows great promise in smart agriculture applications. It offers solutions to various challenges such as crop yield prediction, disease detection, anomaly detection, and weed control. However, there are still challenges to overcome, such as the need for large and diverse datasets, interpretability of models, and addressing data scarcity issues. Future research should focus on addressing these challenges and further exploring the potential of deep learning in smart agriculture.

MATERIAL AND METHODS

Material

Artichoke (Cynara cardunculus) is an edible and medicinal plant with a long history of use dating back to ancient civilizations such as the Egyptians, Greeks and Romans (Acquaviva et al., 2023). It belongs to the Asteraceae family and is known for its distinctive flower heads, which are often consumed as a vegetable. One of the main characteristics of artichoke is its rich phenolic profile, which contributes to its antioxidant properties (Acquaviva et al., 2023). Phenolic compounds such as quercetin and other flavonoids have been detected in artichoke extracts (Wang et al., 2003). It has been indicated that these compounds have inhibitory effects on oxidative stress and may protect against liver damage caused by alcohol consumption (Tang et al., 2017). Artichoke extracts have also revealed potential anti-cancer properties by demonstrating pro-apoptotic activity in colon cancer cells (Villarini et al., 2021). Artichoke is valuable for its nutritional content as well as its antioxidant properties. Studies have evaluated the nutritional value of artichoke heads and reported high levels of vitamins, minerals and bioactive compounds (Petropoulos et al., 2018). Artichoke leaves contain several bioactive compounds, including cynarin, which has been studied for its potential cholesterol-lowering effects (Tang et al., 2017). Analytical methods such as colorimetric analyses, thin layer chromatography and high-performance chromatography have been used to analyze phenolic compounds in artichoke leaves (Wang et al., 2003). These methods provide valuable information about the chemical composition of artichokes, enabling the identification and quantification of specific phenolic compounds. In general, artichoke is a versatile plant with potential health benefits due to its antioxidant properties, nutritional content, and bioactive compounds. More research is needed to explore its therapeutic potential and understand the mechanisms underlying its beneficial effects. Artichoke is a type of vegetable whose underground stem is perennial and its above-ground organs are annual, and whose head and leaves are utilized in various ways. The edible part of the artichoke is the large and fleshy floral receptacle of its unopened flowers and the fleshy bottom parts of the artichoke head, which we call bracts. In addition to fresh consumption, it is canned and deep frozen (Anonymous, 2023a). Artichoke harvesting should be done when the heads have reached their normal size but the bracts have not opened. If the harvest is delayed, the bracts open, the floral receptacle gains a fibrous structure and loses its market value. The heads that reach harvest maturity should be harvested with stems 5 to 10 cm long. After harvesting the artichoke heads, the branches and leaves of the plant dry up and the plant enters a resting period, and no processes are performed during this period. Depending on the region, wake-up irrigation is done in July and August. The years with the highest artichoke production are the 3rd and 4th years. Therefore, it is recommended to renew the plantation after the 4th and 5th years. The number of fruits per plant varies between 3-4 (Anonymous, 2023b).

Method

While preparing the dataset of artichoke vegetable, which was targeted for object detection and analysis within the project, harvest photos and videos taken in the fields of the producers in Tekirdağ Naip Village were used. Sample photos are given in Figure 1. Many artichoke images taken during harvest and growth in the vineyard were collected. Among the images obtained, the images that we could not evaluate within the scope of our project were eliminated. We identified 150 images that would be reliable for our object detection study. In addition, there may be more than one artichoke in each image.

Figure 1. Examples of photos taken in the producer's field in Tekirdağ Naip Village

Labeling

The essential element in an object detection model is the labeling of the objects to be used in the training set. Visual labeling was done on Roboflow, a popular website. RoboFlow is a platform used in various research studies and

applications. It acts as a source for datasets and helps to pre-process and manage image data. In general, Roboflow provides access to datasets and facilitates the pre-processing and management of image data. It can perform field selection, marking and class labeling on the data images to be processed. This marking and labeling process is easily done through the graphical user interface provided by the website. 100 images were used for the training set. In each image, the parts containing the artichoke image were marked with the bounding box area and assigned to the object class "artichoke". The other images and videos were used in the test set. Label Screen is shown in Figure 2.

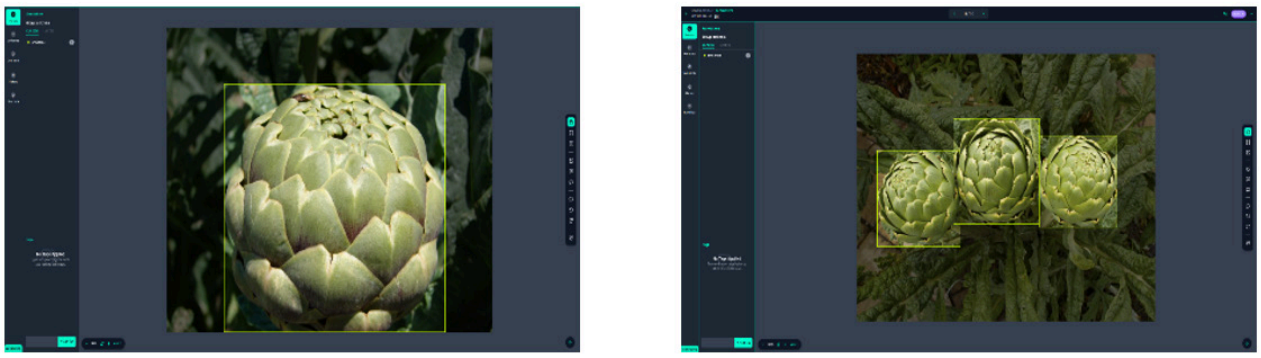


Figure 2. Labeling Screen

Training Model Selection

YOLOv5, one of the deep learning models, was preferred in our study. YOLOv5 is a popular single-stage deep learning algorithm used for object detection. It uses convolutional neural networks (CNN) to detect and classify objects in images. The YOLOv5 algorithm's lightweight structure, improved architecture, and balance between accuracy and speed make it suitable for a variety of applications. YOLOv5 algorithm performs extremely fast processing. It sees the entire image during the training and testing of the data set. Thus, it implicitly encodes contextual information about classes and their views. YOLOv5 learns generalizable representations of objects, and thus shows better performance than other best detection methods when trained and tested on natural images (Nasrullah et al. 2021). YOLOv5 was chosen due to its versatility of usage areas, object detection speed and successful applications in fruit sample segmentation. In the study, YOLOv5n/s/m and l (nano-small-medium and large) models were used in deep learning training. The Yolov5 network structure is shown in Figure 3.

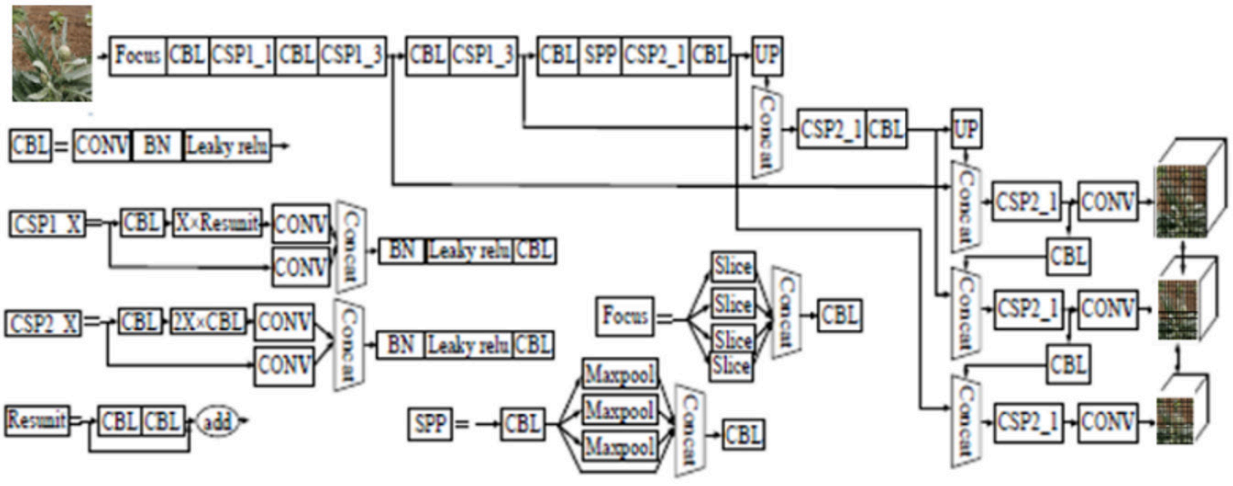


Figure 3. YOLOv5 network structure

Initiation of Training

The training was done with program codes written in the Python ditor with the codes downloaded from GitHub repository, the official site of YOLOv5. The parameters and regulations in the codes written below were preferred for training.

Python train.py –img 640 –batch 20 –epochs 90 –data dataset.yaml –weights yolov5n.pt

python train.py –img 640 –batch 20 –epochs 90 –data dataset.yaml –weights yolov5s.pt

python train.py –img 640 –batch 20 –epochs 90 –data dataset.yaml –weights yolov5m.pt

python train.py –img 640 –batch 20 –epochs 90 –data dataset.yaml –weights yolov5l.pt

-- **img**: The pixel size at which the images to be trained will be reduced by the YOLOv5 model. Its default value is 640x640, and it was chosen in this way here as well.

-- **batch**: The number of data point packets to be used by the display card at a time while training the model.

-- **epochs**: The number of times that all training data is shown to the trained network and the weights are updated while training the model.

-- **data**: The path to the .yaml file containing the general path and class information of the file containing the dataset.

-- **weights**: The location of the weight file containing the training coefficients to be used in training the model.

As a result of running these code lines, the training process of the models has started. The program first checks the YOLOv5 files and checks for any update status. Then, the training process is carried out during the determined number of cycles (epoch).

Evaluation Indicators

Three types of evaluation indicators are generally used to evaluate the model in the field of target detection: Precision, Recall and mAP (mean Average Precision) which is a combination of the first two. Precision represents the total amount of information obtained, that is, the rate of positive samples among all samples in the detection results. TP (True Positive) indicates that both the detection result and the true value are artichoke; FP (False Positive) indicates the number of samples marked as false but detected as positive samples, i.e., the number of false artichokes detected; FN (False Negative) indicates the number of samples marked as positive but predicted as negative classes, i.e. the number of detected artichokes missed. The larger the mAP value, the better the algorithm detection effect and the higher the pattern recognition performance. Here;

Accuracy is the rate of correct classifications/predictions to the total amount of data. Although the problem with the accuracy metric approaching 1 can be stated as successful, it is not sufficient to comment only on this metric.

Accuracy=(TN+TP)/((TP+FP+TN+FN))

Error Rate is the rate of frequency of incorrect classifications/predictions in the problem.

Error Rate=(FN+FP)/((TP+FP+TN+FN)) or (1-Accuracy)

Precision measures model accuracy in artichoke detection by determining the rate of TPs to the total number of predictions made by the model. It is the rate of the positive predictions made in the problem to the accurately positive ones, in other words, the correct ones. The precision value can be calculated using the equation given below.

Precision=TP/((FP+TP))

Recall evaluates the model's ability to correctly identify artichokes among all positive targets, including FN detections or those initially missed and undetected. The equation that calculates how many of the observations that should be predicted correctly are predicted correctly is given below.

Recall=TP/((TP+FN))

F1-Score: It is a metric that can be used instead of accuracy and is very important in terms of interpreting and observing the problem. It is the score resulting from the harmonic average of the Precision and Sensitivity metric values.

F1 Score=(2*Precision)/((Precision+Recall))

mAP: Recall and Precision exhibit a trade-off visualized as a curve by adjusting the artichoke's classification threshold. The area under this recall-precision curve represents the average precision for the artichoke in the model. Averaging these values for all defined classes gives the mean average precision (mAP) that can be calculated. The equation used for the average precision is given below.

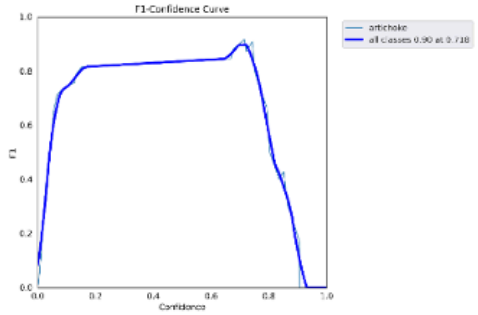
$$mAP = \frac{1}{C} + \sum_{k=1}^{T} P(k)\Delta R(k)$$

RESULTS

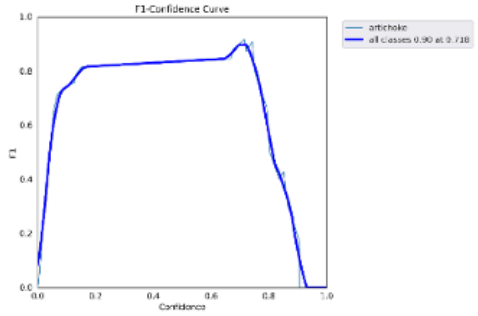
Examination of the results of YoloV5 algorithms according to error matrix metrics

F1 Score

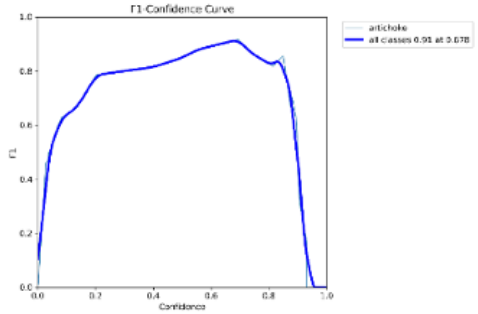
Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5s



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5m



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5l

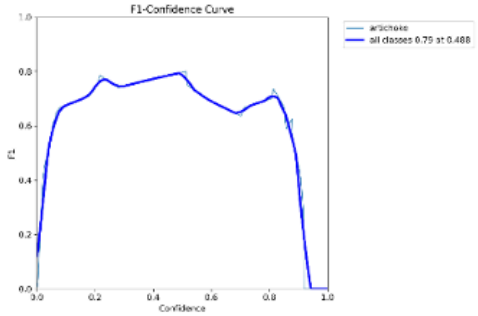
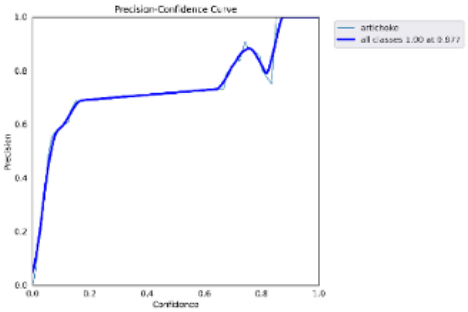


Figure 4. F1 performance score graphs of Yolov5 models

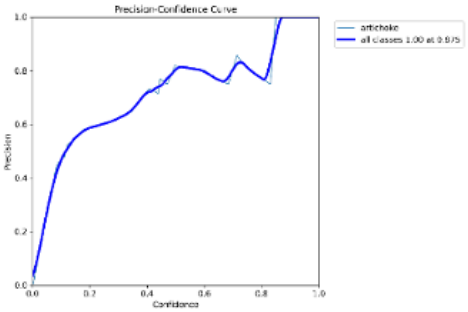
Model 1: F1 score measures the balance between accuracy and false positive rate. This score takes a value between 0 and 1, and the closer it is to 1, the better the performance of the model. The F1 score in the image was determined as 0.85 and indicated that the model had a good performance.

Precision

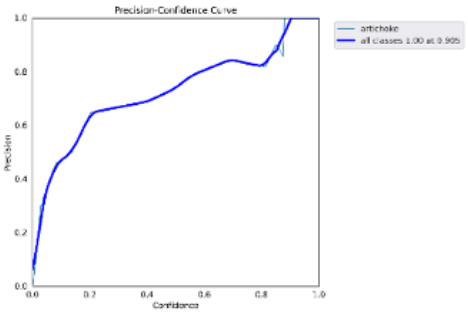
Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5s



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5m



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5l

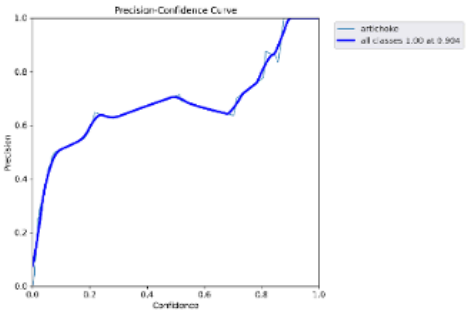
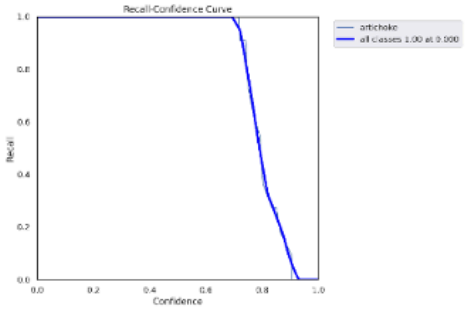


Figure 5. Analysis graphs of precision values obtained in object detection of YOLOv5 models

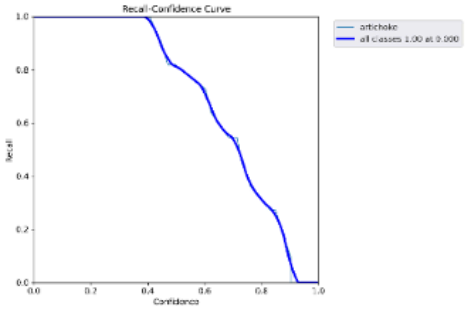
Model 1: The Precision score indicates that approximately 85% of the model's positive predictions are actually positive. This high precision value indicates that the model's positive predictions are mostly true and the rate of false positives is low.

Recall

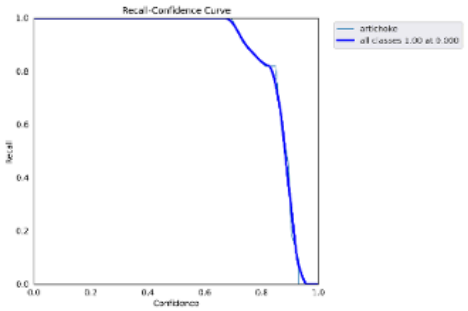
Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5s



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5m



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5l

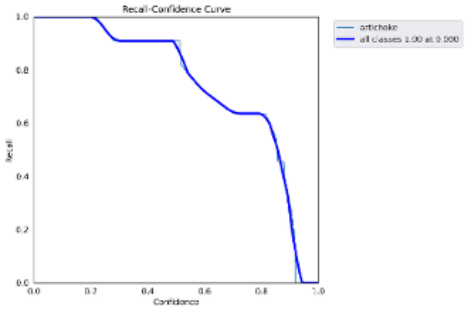
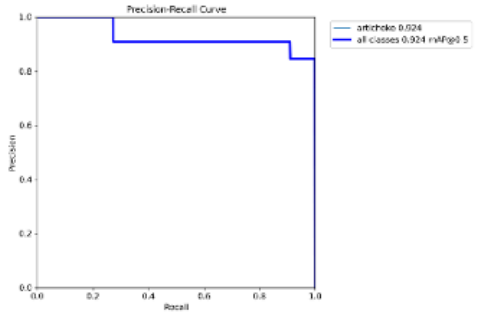


Figure 6. Analysis graphs of recall values obtained in object detection of YOLOv5 models

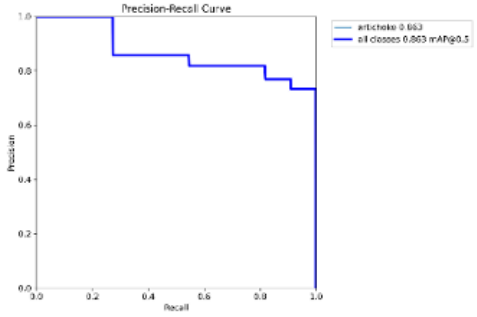
Model 1: A high Recall value shows that the model does not miss the positive class and has a high ability to capture true positives.

Precision Recall

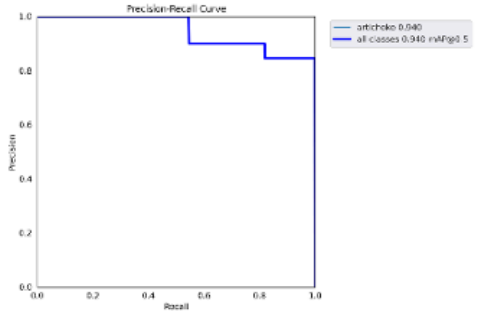
Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5s



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5m



Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5l

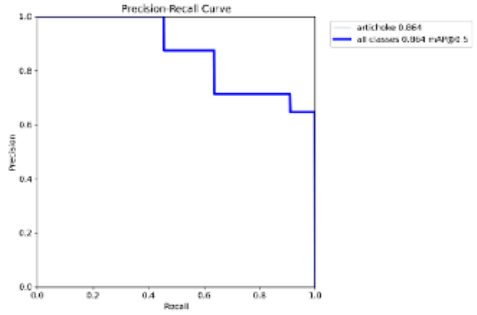


Figure 7. Analysis graphs of precision and recall values obtained in object detection of YOLOv5 models

Model 1: According to the Recall score, the model successfully predicts the majority of true positives. A high Recall score indicates that the precision of the model is high. High Recall usually comes with lower Precision and the number of false positives may increase. Therefore, there should be a balance between Precision and Recall when evaluating the performance of the model.

Loss Function

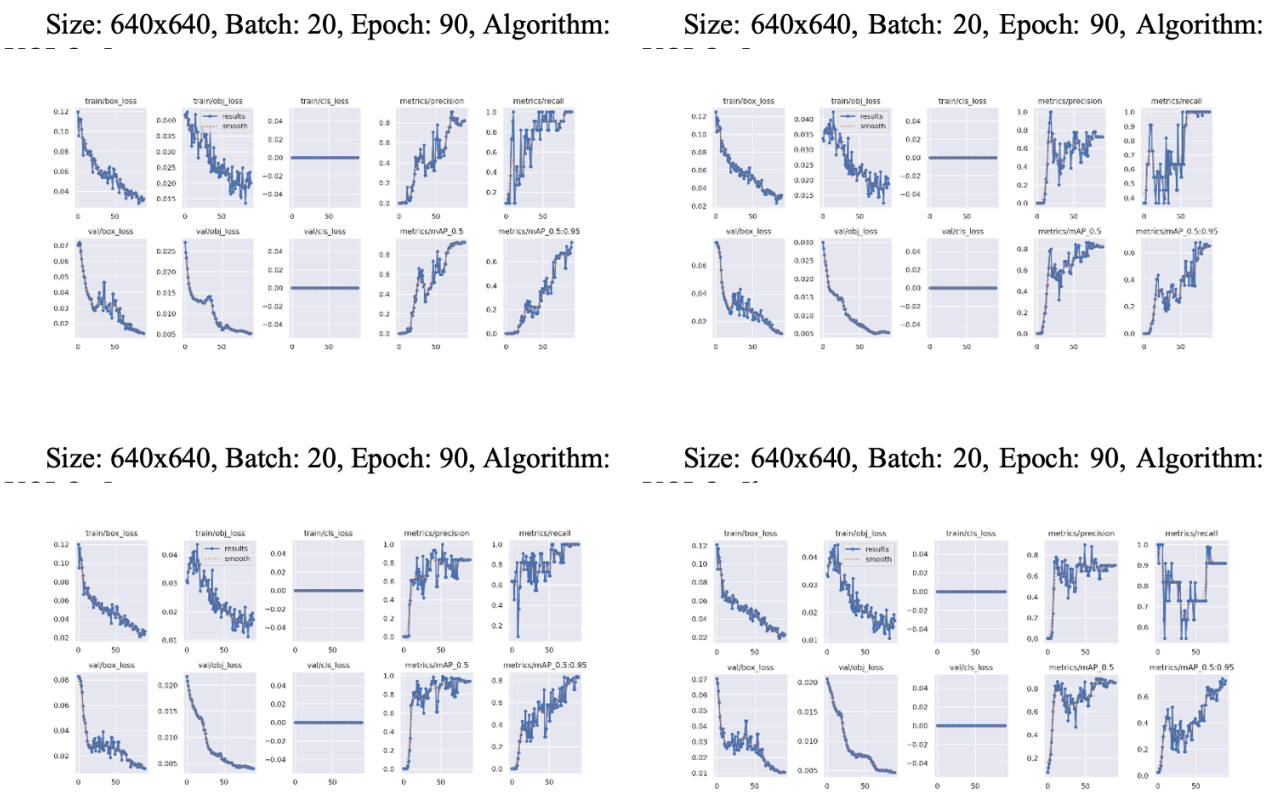


Figure 8. Graphs of error rates and performance values of YOLOv5 models

Model 1: It can be seen that the errors of the model generally decrease over time. This means that the model generally gets better during the training process and its predictions become closer to the true values. However, it seems that the graph fluctuates at certain stages. Therefore, although the overall trend is positive, it is important to identify strategies to further improve the performance of the model and overcome situations that cause these high error rates.

These models differ primarily in their size and complexity, which affect their speed and performance. A comparison based on training results is given below.

Model 1: YOLOv5 NANO

Precision (metrics/precision): 0.81905

Recall (metrics/recall): 1

Average Precision (metrics/mAP_0.5): 0.92388

Tightly Constrained Average Precision (metrics/mAP_0.5:0.95): 0.76145

This model has the highest values in precision and recall metrics, but the tightly constrained average precision is lower.

Model 2: YOLOv5 SMALL

Precision (metrics/precision): 0.7269

Recall (metrics/recall): 1

Average Precision (metrics/mAP_0.5): 0.81932

Tightly Constrained Average Precision (metrics/mAP_0.5:0.95): 0.6499

This model has slightly lower values in precision and recall metrics, but still performs well.

Model 3: YOLOv5 MEDIUM

Precision (metrics/precision): 0.83338

Recall (metrics/recall): 1

Average Precision (metrics/mAP_0.5): 0.94031

Tightly Constrained Average Precision (metrics/mAP_0.5:0.95): 0.82572

This model has the highest precision and recall values at epoch 85. It also has high values in the average precision and tightly constrained average precision metrics.

Model 4: YOLOv5 LARGE

Precision (metrics/precision): 0.7017

Recall (metrics/recall): 0.90909

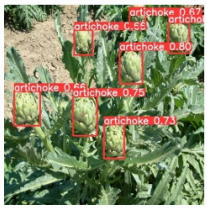
Average Precision (metrics/mAP_0.5): 0.86324

Tightly Constrained Average Precision (metrics/mAP_0.5:0.95): 0.72314

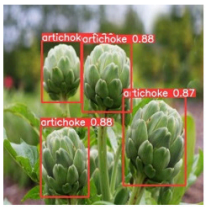
Although this model has lower average precision at epoch 85, the recall value is high. The tightly constrained average precision metric is also high.

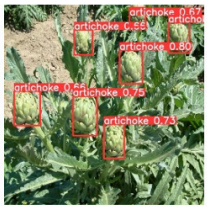
Training Result

Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5n

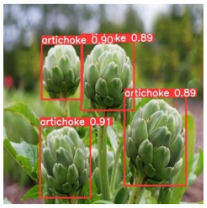


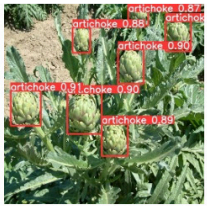
Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5s





Size: 640x640, Batch: 20 Epoch: 90, Algorithm: YOLOv5m





Size: 640x640, Batch: 20, Epoch: 90, Algorithm: YOLOv5l

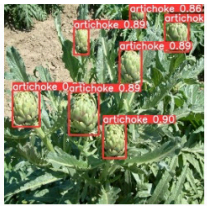


Figure 9. Validation Batch" prediction markings resulting from the training of the models

Comparison of Model Algorithms

The metric data of Model "1" and the difference of other models to these data are shown in Table 1.

Table 1. The metric data of Model "1" and the difference of other models to these data

Model	metrics/precision	Difference (Model 1)	Model	metrics/recall	Difference (Model 1)
Model 1	**0.81905**		**Model 1**	**1**	
Model 2	0.7269	0.09215	Model 2	1	0
Model 3	0.83338	-0.01433	Model 3	1	0
Model 4	0,7017	0.11735	Model 4	0.90909	0.09091
Model	**metrics/mAP_0.5**	**Difference (Model 1)**	**Model**	**metrics/mAP_0.5:0.95**	**Difference (Model 1)**
Model 1	**0.92388**		**Model 1**	**0.76145**	
Model 2	0.81932	0.10456	Model 2	0.66264	0.11155
Model 3	0.94031	-0.01643	Model 3	0.78656	-0.06427
Model 4	0.85324	0.07064	Model 4	0.73866	0.03831

Model 1 has a precision of 0.81905 and a recall value of 1, indicating that it achieves a high precision when capturing all positive samples. Model 2 has a lower precision of 0.7269, indicating a higher false positive rate compared to Model 1. However, it has the same recall value of 1, suggesting that it captures all positive samples. Model 3 has a precision of 0.83338, slightly higher than Model 1, but has a lower recall of 1, indicating that it may miss some positive samples. Model 4 has a precision of 0.7017 and a recall rate of 0.90909, indicating a lower precision and a higher false positive rate compared to Model 1.

In addition to precision and recall, average mean precision (mAP) is another important metric to evaluate the performance of the model. The mAP value of Model 1 is 0.92388, indicating a high average precision between different thresholds. Model 2 has a slightly lower mAP value of 0.81932, indicating a lower average precision compared to Model 1. Model 3 has a higher mAP of 0.94031, indicating a higher average precision. Model 4 has a mAP of 0.85324, indicating a lower average precision compared to Model 1.

Overall, Model 1 performs consistently well in terms of precision, recall, and mAP, and shows its efficiency in capturing positive samples and minimizing false positives. Model 2 shows a decrease in precision compared to Model 1, while Models 3 and 4 have varying performance in terms of precision and recall. However, it is seen that the model with the least loss values in box estimation and object detection losses in the validation data is "Model 1". Training data comparisons of the models are given in Table 2.

Table 2. Comparison of the models according to the training data

Model	train/box_loss	Difference (Model 1)	Model	train/obj_loss	Difference (Model 1)
Model 1	**0.03247**		**Model 1**	**0.020163**	
Model 2	0.032098	0.000372	Model 2	0.018794	0.001369
Model 3	0.026736	0.005734	Model 3	0.017179	0.002984
Model 4	0.023352	0.009118	Model 4	0.017004	0.003159
Model	val/box_loss	Difference (Model 1)	Model	val/obj_loss	Difference (Model 1)
Model 1	**0.013598**		**Model 1**	**0.0051987**	
Model 2	0.011416	0.002182	Model 2	0.0052638	-0.0000651
Model 3	0.0097465	0.0038515	Model 3	0.0040057	0.001193
Model 4	0.010439	0.003159	Model 4	0.0046527	0.000546

The differences in "train/box_loss" and "train/obj_loss" between Model 1 and other models can be attributed to the use of different loss functions. The fact that Model 1 has a higher loss value compared to other models indicates that it may have a less effective loss function for training. On the other hand, the fact that Models 2, 3, and 4 have lower loss values indicates that the loss functions are more effective in minimizing the difference between predicted and ground truth bounding boxes and object classes.

Model 1 also has higher loss values than other models in terms of "val/box_loss" and "val/obj_loss". This reveals that Model 1 performs worse in terms of object detection and classification in the validation set. The fact that the Models

2, 3, and 4 have lower loss values reveals that they perform better in accurately locating and classifying objects in the validation set.

In general, differences in loss values between Model 1 and other models can be attributed to the choice of loss functions. Models 2, 3, and 4, which have lower loss values, probably use more efficient loss functions such as GH-SSD loss [1] or MC-Loss [2]. These loss functions help to improve the training process and improve the model's ability to accurately detect and classify objects.

Finally, the optimization parameters (x/lr0-1-2) of the models were examined. All models have equal values in these parameters. Parameter values are given in Table 3.

Table 3. Optimization parameters of the models

Model	x/lr0-1-2	Difference (Model 1)
Model 1	0.00032	
Model 2	0.00032	0
Model 3	0.00032	0
Model 4	0.00032	0

The object detection accuracies of YOLOv5 models and sample training and validation images made with the prepared data set were examined. When the metric data and accuracy prediction rates indicating the object detection success of the models were examined, it was confirmed that the training result of the "YOLOv5 nano" model was more successful than the others.

DISCUSSION

In their study, Zhang et al. (2022) performed weed-crop classification and lettuce localization in the field using the SE-YOLOv5x deep learning model. As a result of the study, SE-YOLOv5x had the highest performance in weed and lettuce plant identification with 97.6%, 95.6%, 97.1% and 97.3% precision, recall, mean precision (mAP) and F1-score values, respectively. Based on plant morphological characteristics, the SE-YOLOv5x model found the location of the lettuce stem in the field with 97.14% accuracy. Yang et al. (2022) designed a blueberry recognition model based on YOLOv5. To verify the efficiency of the model, the mAP on the blueberry dataset in the study was 83.2%, which was 2.4% higher than the original network. This proved that the proposed method was useful in increasing the blueberry recognition accuracy of the model. Su et al. (2022) performed a tree trunk and obstacle detection method in a semi-structured apple orchard environment based on the improved YOLOv5s to improve real-time detection performance. As a result of the study, they found the average precision values of the model in spring, summer, autumn and winter as 95.61%, 98.37%, 96.53% and 89.61%, respectively. The model size of the developed model was reduced by 13.6 MB, and the accuracy and average accuracy on the test set were increased by 5.60% and 1.30%, respectively. The average detection time was 33 ms and they determined that an orchard carrier platform would meet the real-time detection requirements. In their study, Fu et al. (2022) investigated the control technology for targeted spraying by applying deep learning and online identification methods with cabbages as the research object. To overcome motion blur and low average precision under strong light conditions during the running of the sprayers, they used an innovative YOLOV5 model embedded with a transformer module to obtain accurate online identification for cabbage fields in complex environments. They determined that the target-oriented spraying system designed in the study achieved the expected experimental results and could provide technical support for field target spraying. Wang et al. (2022) developed a cucumber root-knot nematode detection model based on the modified YOLOv5s model to support the breeding of robust cucumber varieties. In the experimental results, they found that the recall (R) and mAP values of the YOLOv5s-CMS model were improved by 3% and 3.1%, respectively, compared to the original YOLOv5s model. They emphasized that with these values, the model could achieve a better performance in detecting cucumber root-nematode. Wu et al. (2022) conducted a study on the problem of automatic classification of horn mushrooms. To solve the problem, they deeply integrated YOLOv5's single-stage object detection with PSPNet's semantic segmentation and constructed a Y-PNet model and an image segmentation network for real-time object detection. As a result of the experiments, they emphasized that the system can successfully perform real-time grading, which can provide instructive and practical references in the industry. Rong et al. (2022) designed a special end effector for robotic harvesting, which mainly consisted of a flexible gripper and a cutting device to grasp the fruits and cut the pedicles. With the YOLOv5s-CBAM model they developed, they found watermelon fruits with 89.8% accuracy in the test dataset. The overall harvest success rate was 85.0% with positioning error. López-Correa et al. (2022) evaluated a new method to automatically detect and classify the most problematic weed species in tomato crops in one step. The procedure is based on object detection neural networks called RetinaNet. They also evaluated two existing mainstream object detection models, YOLOv7 and Faster-RCNN, as a one- and two-stage NN, respectively, in comparison with RetinaNet. As a result of the experiments, the prediction model was validated with images that were not used during training

under the average precision (mAP) metric. RetinaNet showed an AP value ranging from 0.900 to 0.977, depending on the weed type. Faster-RCNN and YOLOv7 also achieved satisfactory results in terms of mAP, especially through data augmentation. Xie et al. (2022) developed an improved litchi detection model called YOLOv5-litchi for litchi detection in complex natural environments. As a result of the study, they found that the mAP and recall values of the YOLOv5-litchi model were improved by 12.9% and 15%, respectively, compared to those of the unimproved YOLOv5 network. They found that the inference speed of the YOLOv5-litchi model to detect each image was 25 ms, and they emphasized that this speed was much better than Faster-RCNN and YOLOv4. Fu et al. (2022) developed a dynamic detection method based on an improved YOLO-v5 network for an accurate broad bean phenotype definition. In the experimental phase, they analyzed the effect of different data sets on the model and the performance of different models on the same data set under the same test conditions. When the test results were compared with the network models trained on the RGB dataset, they found that the recall and precision of the models trained on the RGB-D dataset increased by approximately 32% and 25%, respectively. As a result of comparison with YOLO-v5s, they found that the precision of the improved YOLO-v5 increased by approximately 6%, with a precision of 88.14% for the determination of the amount of broad bean with 200 plants in the soybean population.

This study was conducted to determine the best deep learning detection model that can be used for robotic harvesting of artichokes. The YOLOv5 model was also used successfully in the current studies we mentioned. It was observed that the results in the studies were parallel to the results found in the study. The study indicated the potential of using crop identification for robotic systems in artichoke harvesting. All metrics of YOLOv5 were examined to increase the performance of robotic systems to be designed for use in artichoke harvesting. It was observed that such performance evaluations will be of great importance for the development and optimization of robotic harvesting systems.

CONCLUSIONS

In this study, YOLOv5 deep learning model was used to detect artichoke on seedlings. All sub-algorithms of the model were applied in the study. The detection success of the models on artichoke seedlings was examined as metric and validation prediction values. It was determined which sub-model of YOLOv5 gave the best results according to the success rates in object detection, metric and verification prediction values. According to loss values and learning speed rates, the best sub-algorithm model was confirmed as YOLOv5nano. The training of each sub-model was performed as Size: 640x640, Batch: 20, Epoch: 90. As a result of the testing phase, it was seen that the value python train.py --img 640 --batch 20 --epochs 90 --data dataset.yaml --weights yolov5n.pt gave the best results. It was understood that the higher Precision value of the YOLOv5n model compared to other models increased the success of the model. The information obtained from this research will greatly facilitate the detection and robotic harvesting of artichokes on seedlings.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

All data associated with this research were indicated and used in the manuscript submitted.

Consent to participate

Not applicable.

Consent for publication

All authors consented to the publication of this manuscript.

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RESEARCH ARTICLE

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Optimization of bioactive components of ultrasound treated white grape juice

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Abstract

Grapes are among the most commonly produced fruits worldwide, thanks to their ease of cultivation in terms of climate and soil requirements. Owing to their abundant vitamins, bioactive compounds and minerals incorporating them into one's diet is advised for promoting human health. The bioactive components of ultrasound-treated white grape juice were optimised by response surface methodology (RSM). In addition, untreated white grape juice (C-WGJ), ultrasound treated white grape juice (U-WGJ) and thermally pasteurized white grape juice (P-WGJ) samples were compared for total phenolics compound (mg GAE/L), total flavonoids (mg CE/L) and DPPH (% Inhibition) parameters. The scientific investigation employed RSM, a widely favored approach. During the study, ultrasound process parameters such as amplitude (40, 50, 60, 70 and 80%) and duration (2, 4, 6, 8 and 10 minutes) were manipulated. The responses to the process application were assessed through analyses of total phenolic content (TPC), total flavonoid content (TFC) and DPPH (1,1-Diphenyl-2-Picryl Hydrazyl). The model achieved commendably high R^2 values following optimization through RSM. One-way ANOVA for DPPH value, TFC and TPC of samples, both amplitude and duration exhibited statistically significant effects ($p<0.001$). The effectiveness of ultrasound treatment in increasing bioactive components in untreated white grape juice is higher than thermal pasteurization treatment. Through the optimization process, the best bioactive values for white grape juice were achieved, measuring 42.3 mg CE/L for TFC, 55.5% inhibition for DPPH and 440.3 mg GAE/L for TPC. Ultrasound applied to white grape juice proved to be more effective in preserving and enhancing bioactive compounds than thermal pasteurization. Consequently, the findings suggest the need for further investigations to assess the impacts of both thermal pasteurization and ultrasound technology on additional quality parameters of white grape juice.

Keywords: Bioactive substances, RSM, Ultrasound

INTRODUCTION

Globally, grapes stand out as the most extensively grown fruits, appreciated for their adaptability to various climates and soil conditions. Their categorization as "red" or "white" hinges on the presence of anthocyanins in their outer skin (Georgiev et al., 2014). Cultivated across continents, grapes thrive in regions boasting adequately, warm, rainy and dry summers, coupled with relatively mild winters. Turkey, given its climatic conditions, emerges as a bountiful reservoir of grapes (Vivier & Pretorius, 2000). In their skin, white grapes harbor flavonoids (isorhamnetin-3-O-glucoside, quercetin 3,4'-O-glucoside, kaempferol-3-O-glucuronide, quercetin 3-O-glucoside, quercetin 3-O-glucuronide, and

kaempferol-3-O-glucoside) and flavanols (proanthocyanidins, catechin, epicatechin) with a particular emphasis on the richness of these compounds (Sabra et al., 2021). Economically attainable and delectable, grapes are advocated as a healthful dietary choice for humans, given their abundance in bioactive compounds, minerals, and vitamins (Fernandes et al., 2017). Anticarcinogenic, anti-obesity, antiviral, and antioxidant effects are manifested by bioactive components (Zhou et al., 2022).

The objective of food processing is to generate products that are more convenient, attractive, value-enhanced, and possess an extended shelf life. The conventional approach to food processing primarily employs heat-based methods to manage the development of foodborne pathogens and curtail microbial growth. However, when applied to foods sensitive to heat, these procedures may induce alterations in color, taste, and consistency (Singla & Sit, 2021). In lieu of thermal methods, cutting-edge technology, specifically ultrasound-assisted processing has been devised. This approach is recognized as a health-conscious, environmentally friendly, secure and non-thermal method for physical processing (Zhang et al., 2023). Numerous scholars have determined that employing non-thermal methods, such as ultrasound treatment, results in minimal diminishment of nutritional value and quality across a range of food products. These include tangerine juice (Tokatlı Demirok & Yıkmış, 2022), cranberry vinegar (Erdal et al., 2022), pineapple juice (Hoque et al., 2022)including physicochemical properties, antioxidant activities, and microbial inactivation, were studied. Pineapple juice was sonicated at 100 W and 140 W (for 5, 10 and 15 min, each and apricot juice (Sattar et al., 2020)microwaved for 1.5 min at 850 W of power and sonicated for 90 min at 20 kHz of frequency were selected to keep in storage for up to 30 days in refrigerator to examine the changes happened to their physicochemical characteristics and functional components. It was observed that the pH and the cloud values of all processed juice samples reduces with the storage time, whereas, the total soluble solids almost remain consistent particularly in microwave and ultrasound treated samples. While storage period causes the decrement in total phenolic content (TPC, rosehip nectar (Atalar et al., 2020), black grape juice (Yıkmış et al., 2023) and guava juice (Kalsi et al., 2023).

RSM is a statistical approach that demands a reduced investment of time and effort. It has been consistently and effectively applied for the continuous optimization of various contents (Ruby-Figueroa et al., 2023). RSM is a popular technique for multivariate statistical methods. RSM is a persistent system combination based on the fit to the stored polynomial model, which should result in the storage of all data for the purpose of fitting the expanding model for predictions (Ghorbannezhad et al., 2016). Following a comprehensive review of the existing literature, no investigations were identified pertaining to the optimization of bioactive components in white grape juice through RSM. The main objective of the study was to optimise the bioactive components (TPC, TFC, DPPH) of ultrasonically treated white grape juice using RSM and to produce white grape juice rich in bioactive components. At the same time, untreated white grape juice (C-WGJ), ultrasonically treated white grape juice (U-WGJ) and thermally pasteurised white grape juice (P-WGJ) samples will be compared in terms of TPC, TFC, DPPH parameters.

MATERIALS AND METHODS

Preparation of white grape juice samples

In the research, fresh white grapes were carefully harvested from Tekirdag, Turkiye. The juice was crafted by utilizing a blender (Waring Commercial Blender Model HGB2WTS3, USA) to crush the grapes. Following filtration through a double-layer cheesecloth, the resultant white grape juice underwent sterilization and was then bottled in 200 mL airtight containers. The untreated white grape juice served as the control (C-WGJ). Another batch of white grape juice underwent pasteurization in a water bath at 90 ˚C for 30 seconds, with subsequent rapid cooling to 20 ˚C (P-WGJ). Additionally, a 200 W ultrasonic processor (Hielscher Ultrasonics Model UP200St, Berlin, Germany) operating at a frequency of 26 kHz was employed to process another 200 mL of white grape juice (U-WGJ). Maintaining temperature control throughout the ultrasound procedure was achieved through the utilization of an ice bath. Subsequently, all specimens were preserved at -18 ˚C until the analytical stage.

Experimental structure

Leveraging RSM, the fine-tuning of bioactive constituents in the white grape variant has been conducted using ultrasound technology, known for its superior attributes over thermal pasteurization. The ultrasound procedure encompassed variables including duration minutes (2, 4, 6, 8, 10) and amplitude (40%, 50%, 60%, 70%, 80%). Analyses of TPC, TFC and DPPH were executed as responses to the application of the process. A Central Composite Design was opted for and a two-factor, five-level experimental arrangement was formulated (Table 1). A total of 13 experimental setups were devised for this investigation. Adequacy values of the model were assessed through lack-of-fit tests, ANOVA results, and the consideration of R^2 and adjusted -R^2 coefficients. The independent variables were defined within the temporal (X_1) and amplitude (X_2) ranges.

Table 1. The dependent and independent parameters of the RSM assay and the results of bioactive compounds

Sample[a]	Encoded Independent Variables		Dependent Variables					
			Response 1		Response 2		Response 3	
	Time (X_1)	Amplitude (X_2)	Total Phenolics Compound (mg GAE/L)		Total flavonoids (mg CE/L)		DPPH (% Inhibition)	
			Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	8	50	440.48±1.05	440.06	41.77±1.73	41.72	54.60±0.86	54.48
2	8	70	439.83±0.44	439.29	42.35±0.31	42.34	55.50±0.52	55.46
3	6	60	438.16±1.86	438.26	41.78±0.79	41.68	54.60±1.95	54.70
4	2	60	427.44±1.50	426.87	41.13±0.91	41.08	53.10±0.48	53.10
5	6	60	438.16±1.41	438.26	41.68±0.11	41.68	54.80±1.13	54.70
6	6	60	438.16±0.23	438.26	41.66±0.62	41.68	54.72±2.12	54.70
7	6	60	438.27±0.71	438.26	41.66±0.72	41.68	54.72±0.4	54.74
8	10	60	438.45±0.64	438.99	42.50±0.38	42.53	54.64±0.72	54.70
9	4	70	437.75±1.85	438.34	41.59±0.68	41.67	53.76±1.07	53.81
10	6	80	441.41±1.37	441.47	42.22±0.79	42.19	55.04±0.88	55.03
11	4	50	428.17±0.85	428.89	40.90±0.35	40.94	54.48±0.54	54.51
12	6	60	438.58±2.23	438.26	41.66±1.41	41.68	54.80±0.25	54.74
13	6	40	432.89±1.90	432.79	40.82±1.09	40.84	54.74±0.44	54.76
C-WGJ			425.76±1.07		40.18±0.24		52.18±0.65	
P-WGJ			410.44±0.79		38.55±1.22		49.65±0.57	
U-WGJ-Multiple Response Prediction	6.8 min	%80 amplitude	440.30		42.30		55.50	
Experimental values			435.26±1.78		41.15±0.92		53.44±0.28	
% Difference			1.14		2.72		3.71	

RSM: Response surface methodology C-WGJ: white grape juice P-WGJ: thermal pasteurized white grape juice U-WGJ: ultrasound-treated white grape juice TPC: total phenolic content; TFC: total flavonoid content; DDPH: radical scavenging activity; GAE: gallic acid equivalent; CE: equivalent of catechins

Determination of total phenolic components

Folin-Ciocalteu method developed by Singleton and Rossi (1965) was used for TPC detection (Singleton & Rossi, 1965). 0.5 mL samples of fruit juice, suitably diluted with distilled water, were withdrawn and 2.5 mL of 0.2 N Folin-Ciocalteau reagent was introduced. Following a 3-minute interval, 2 mL of 7.5% (w/v) Na_2CO_3 solution was incorporated. The vials were then placed in a dimly lit environment at room temperature for 30 minutes. Subsequently, the absorbance value was gauged at a wavelength of 760 nm utilizing a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia). The TPC was quantified in milligrams of gallic acid equivalents per liter (mg GAE/L). The analyses were conducted in triplicate.

Determination of total flavonoid components

TFC was calculated colorimetrically using a UV spectrophotometer (Spectrum Instrument, SP-UV/VIS-300SRB, Australia) (Zhishen et al., 1999). A certain amount (1 mL) of the sample was added to a 10 mL tube containing 4 mL of double-distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added to the tube, and after 5 minutes, 0.3 mL of $AlCl_3$ (10%) was added. At the 6th minute, 2 mL of NaOH (1 M) was added, and the total volume was made up to 10 mL with double-distilled water. The tubes were left in a dark environment at room temperature for 30 minutes. Subsequently, the absorbance value was measured at a wavelength of 510 nm using a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia). The total flavonoid content was expressed as milligrams of catechin equivalents (CE) per liter. The experiments for total flavonoid analysis were conducted in triplicate.

DPPH free-radical scavenging activity

Evaluation of antioxidant activity was carried out using DPPH radical (Grajeda-Iglesias et al., 2016). For 1 mL of white grape juice, 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.2 mM in methanol) was introduced, followed by incubation in the absence of light at room temperature (25 ± 1 ◦C) for 30 minutes. The analyses were conducted in triplicate. Alterations in absorbance were measured at 517 nm using a spectrophotometer (SP-UV/VIS-300SRB, Melbourne, Australia). The outcomes were quantified as % inhibition.

Statistical analysis

All values were obtained in triplicate and expressed as mean ± standard deviation (SD). The RSM was performed using the Minitab software package (version 19. Minitab Software); the RSM plots were developed using the SigmaPlot 12.0 software package (Systat Software. Inc.).

RESULTS AND DISCUSSION

Optimization of bioactive components

Non-thermal pasteurization is a technique devised to mitigate alterations in the nutritional and sensory attributes of food. Serving as a substitute for thermal methodologies, this approach is pioneering and ecologically sustainable (Bhargava et al., 2021; Fan et al., 2021; Valiati et al., 2022). This non-thermal technology offers several advantages such as rapid processes, enhanced process efficiency, elimination of process steps, better quality product and retention of product characteristics (texture, nutrition value, organoleptic properties. White grape juice is a beverage renowned for its elevated antioxidant activity, attributed to the presence of flavonoids and flavanols in its composition. The bioactive constituents in foods can be impacted by elevated temperatures. Due to their high temperatures, thermal treatments can negatively affect the sensory appeal and nutritional quality of food, making them vulnerable to adverse effects. Consequently, aside from preservation, there arises a necessity for the augmentation of bioactive components in white grape juice through non-thermal methods. Another objective of this study is to amplify the bioactive constituents of white grape juice via ultrasound treatment. The optimization outcomes for TPC (mg GAE/L) TFC (mg CE/L) and DPPH (%inhibition) content of white grape juice are delineated in Table 2.

Table 2. ANOVA results of bioactive components (TPC, TFC and DPPH)

		TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (% Inhibition)	
Source	DF	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	5	157.08	0.000	171.49	0.000	152.25	0.000
Linear	2	280.06	0.000	416.05	0.000	192.24	0.000
X_1	1	371.19	0.000	447.65	0.000	373.36	0.000
X_2	1	188.94	0.000	384.45	0.000	11.12	0.013
Square	2	68.72	0.000	12.23	0.005	122.19	0.000
X_1*X_1	1	136.66	0.000	6.03	0.044	197.72	0.000
X_2*X_2	1	6.10	0.043	11.70	0.011	6.54	0.038
2-Way Interaction	1	87.81	0.000	0.88	0.379	132.39	0.000
X_1*X_2	1	87.81	0.000	0.88	0.379	132.39	0.000
Error	7						
Lack-of-Fit	3	19.61	0.007	1.90	0.271	1.43	0.358
Pure Error	4						
Total	12						
R^2		99.12%		99.19%		99.09%	
Adj. R^2		98.49%		98.61%		98.44%	
Pred. R^2		91.53%		94.85%		94.64%	

X_1: time; X_2: amplitude; DF: degrees of freedom; R^2—coefficient of determination; DDPH: radical scavenging activity; TFC: total flavonoid content; TPC: total phenolic content; GAE: gallic acid equivalent; CE: equivalent of catechins; $p < 0.05$, significant differences; $p < 0.01$, very significant differences.

Following the optimization, Formula (1) outlines the second-degree modeling equation for the TPC value in white grape juice, expressed in milligrams of gallic acid equivalents per liter (mg GAE/L).

$$\text{TPC mg GAE/L} = 348.03 + 13.180X_1 + 1.321X_2 - 0.3330X_1X_1 - 0.00281X_2X_2 - 0.1278X_1X_2 \quad (1)$$

Table 2 provides the R^2 values, analysis of variance (ANOVA), lack-of-fit assessment, and regression coefficients. White grapes, an integral part of the Mediterranean diet, are recognized for their health advantages and substantial levels of flavonols and phenolic acids (Montalbano et al., 2021).

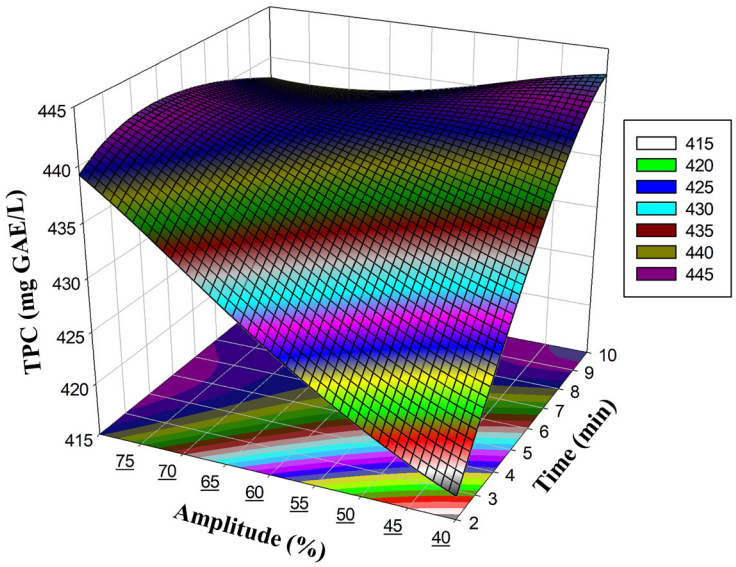

Figure 1. Response surface plots (3D) of TPC as functions of significant interaction factors

Our investigation revealed that ultrasound treatment led to an elevation in the TPC value of white grape juice. A 3.42% enhancement was noted in comparison to the C-WGJ sample. Three-dimensional RSM graphs were generated to pinpoint the optimal values of independent variables for bioactive components (DPPH, TPC, and TFC). The impact of ultrasound on TPC was elucidated through the response surface graph illustrated in Figure 1. Upon examining the impacts of time and amplitude, a consistent augmentation in the TPC (mg GAE/L) mount was observed. The RSM modeling level exhibited a robust fit with an R^2 value of 99.12% (Table 2). Both the one-way and two-way effects of the model were statistically significant ($p<0.05$). Subsequent to the optimization process, the TPC was ascertained to be 440.3 mg GAE/L (Table 1). In a study conducted by Kalsi et al. (2022) investigating the influence of ultrasound on the quality of guava juice, akin to our findings, a noteworthy surge in the TPC (3.50–4.35%) was observed post-ultrasound treatment. ($p < 0.05$) (Kalsi et al., 2023). The thermosonication process treated to black grape juice (*Vitis vinifera L.*) (Yıkmış et al., 2023) has increased the TPC value of grape juice. The findings in these studies are consistent with our study, indicating that thermal pasteurization processes treated similarly to grape juices further reduce the TPC. In other studies comparing ultrasound treatment and thermal pasteurization on fruit juices, it was observed that ultrasound treatment not only protected but also enriched the bioactive components of fruit juices. The fact that the thermal pasteurization process causes a decrease in the amounts of all bioactive components can be explained as the high temperature applied damages the structure of the compounds (Kahraman & Feng, 2021; Tokatlı Demirok & Yıkmış, 2022).

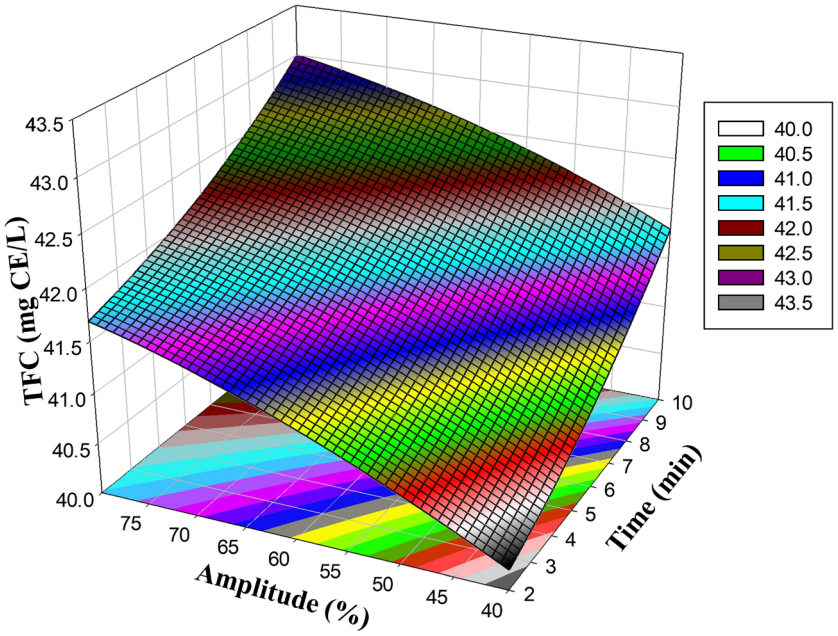

Figure 2. Response surface plots (3D) of TFC as functions of significant interaction factors

Following the RSM, the R^2 value for TFC demonstrated a substantial correlation at 99.19% (Table 2). The second-degree polynomial equation for the enhancement of white grape juice is delineated in Formula (2).

$$TFC(mg\ CE/L)=36.800+0.1744X_1+0{,}0933X_2+0.00765X_1X_1-0.000426X_2X_2-0{,}00140X_1\ X_2 \quad (2)$$

In the one-way (ANOVA) for TFC, both duration and amplitude exhibited statistical significance ($p<0.001$), whereas in the two-way interaction, TFC did not reach statistical significance ($p>0.05$). Subsequent to optimization, the TFC value was established at 42.30 mg CE/L. Post-pasteurization, TFC was measured at 38.55±1.22 mg CE/L. Following ultrasound treatment, a 5.27% rise was noted in comparison to the C-WGJ sample. In the P-WGJ sample, a 4% decrease was observed in contrast to the C-WGJ sample (Table 1). The ultrasound process exhibited superior preservation of the TFC in white grape juice when compared to thermal pasteurization. The impact of ultrasound on TFC is elucidated through the response surface graph depicted in Figure 2. Both the time and amplitude effects were observed to enhance the quantity of TFC (mg CE/L). In a study conducted by Manzoor et al. (2023), analogous to our investigation, an elevation in TFC and TPC values was noted following sonication in the assessment of the impact of sonication on the general characteristics of the quality of the tomato juice (Faisal Manzoor et al., 2023). Tokatlı Demirok et al. (2023) documented that the application of ultrasound elevated the TFC in conventional apple vinegar enriched with horsetail-fortified (Tokatlı Demirok et al., 2023). In their investigation, Yıkmış et al. (2021) observed that the application of ultrasound enhanced the bioactive constituents of cherry laurel (*Prunus laurocerasus*) vinegar, leading to an augmentation in the total flavonoid content in comparison to the untreated vinegar (Yıkmış et al., 2021). In our investigation, there were observed increments in bioactive components. Ultrasound induces the compression and rarefaction of the medium at a specific point, reaching a critical molecular distance. The surpassing of this distance disrupts the medium, giving rise to cavitation bubbles. The heightened pressure and temperatures resulting from these cavitation bubbles close to the cell surface generate microjets that are directed at the cells, causing the breakdown of cellular structures (Chemat et al., 2011; Perera & Alzahrani, 2021).

Following the RSM, the R^2 value for DPPH exhibited a robust correlation at 99.09% (Table 2). The second-degree polynomial equation for the enhancement of white grape juice is provided in Formula (3).

$$DPPH(\%inhibition)=60.122 - 0.412\ X_1 - 0.1655\ X_2 - 0.05351\ X_1\ X_1 + 0.000389\ X_2\ X_2 + 0.02096\ X_1\ X_2 \quad (3)$$

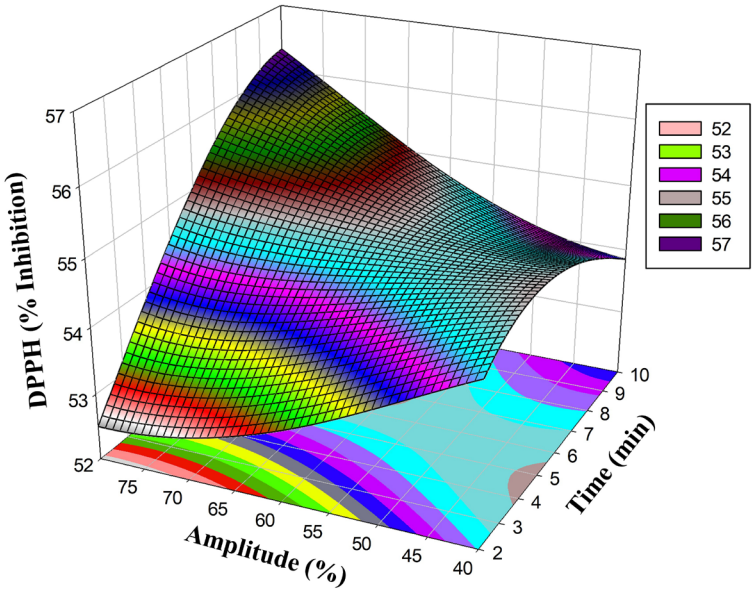

Figure 3. Response surface plots (3D) of DPPH as functions of significant interaction factors

Observations indicate that with the increment in time and amplitude values, there was a corresponding increase in the DPPH (%inhibition). These increments are visually evident in the three-dimensional model graph (Figure 3). Statistically significant disparities were identified in the two-way interaction of the DPPH antioxidant value ($p<0.05$). Broadly, diverse ultrasound treatments showcased an augmentation in the DPPH value of white grape juice. The DPPH value for the U-WGJ sample is 55.50%, the DPPH value for the C-WGJ sample is 52.18±0.65% and the DPPH value for the P-WGJ sample is 49.65±0.57%. While ultrasound processing led to an increase in the DPPH value, thermal pasteurization resulted in noteworthy decreases in the DPPH value. Our study aligns with analogous findings reported in research on Amazon fruits (buriti and açaí) (de Souza Carvalho et al., 2020), apple beverage enriched with blueberry extracts and black carrot (Brezan et al., 2020) peach and apricot juices after ultrasound treatment (Sattar et al., 2020). Sattar et al., (2020) found that microwave (1.5 min at 850 W) and ultrasound (90 min at 20 kHz) treatment caused a decrease in TPC while increasing DPPH.

CONCLUSION

Grapes cultivated in different regions worldwide are renowned for their health-promoting properties and being an integral part of nutrition due to their abundant antioxidants. This research aimed to optimize the bioactive components in white grape juice using RSM and ultrasound treatment. Additionally, the bioactive values of C-WGJ, U-WGJ and P-WGJ samples were compared. Compared to the C-WGJ sample, U-WGJ samples showed increases of 3.42%, 5.27% and 6.36% in TPC, TFC and DPPH values respectively. The increase in concentrations of bioactive compounds in U-WGJ samples could be attributed to the cavitation pressure induced by ultrasound treatment, resulting in the release of bound bioactive compounds due to the breakdown of fruit cell walls and addition of hydroxyl groups to the phenolic aromatic ring. However, a decrease in bioactive values was observed in P-WGJ samples, possibly due to the high temperature applied during thermal pasteurization damaging the structure of components. RSM results indicated a strong correlation between predicted and experimental values. In summary, optimization of process conditions for ultrasonically treated white grape juice resulted in the enrichment of bioactive components, demonstrating the significant impact of this technology on enhancing bioactive components. This technology stands as an alternative to thermal techniques in food processing due to its advantages in increasing bioactive components.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

M.T.: Conception, Design, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Review, Writing, and Critical Review; N.T.D.: Conception, Materials, Supervision, Analysis and Interpretation, Writing, and Critical Review; S.Y.: Conception, Materials, Supervision, Fundings, Writing, and Critical Review; B.İ.A.: Conception, Literature Review, Writing, and Critical Review.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Yield and yield components of some advanced Camelina (*Camelina sativa* L. CRANTZ) genotypes in Bolu ecological conditions

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Abstract

The negative effects of global warming are increasing worldwide and this increase is expected to continue. The camelina plant, which can be grown in marginal areas, stands out among other oilseed plants because it is drought-resistant and less costly. In the research, seeds belonging to 8 camelina genotypes, which were prominent in terms of oil rate, seed and oil yield, were used in the trial established in Bolu province, out of a total of 52 genotypes obtained from the seed bank of the United States Department of Agriculture, Agricultural Research Service and Germany. The study was conducted according to the Randomized Complete Block Design in Bolu Abant İzzet Baysal University, Faculty of Agriculture, Research and Application Field in the 2021-2022 production season. In the trait, each plot consisted of six rows with a row length of 3 m, a row spacing of 20 cm, and in row of 5 cm, with three replications. According to the research results; plant height 66.33-71.00 cm, number of branches 3.17-5.07 number plant^{-1}, 1000 seed weight 1.10-1.24 g, seed yield 1095.4-1436.6 kg ha^{-1}, oil content 36.63-40.37%, protein content 23.65-27.22%, oil yield 408.3-559.8 kg ha^{-1} and protein yield 279.0-391.3 kg ha^{-1}. It was found that values between. According to the results obtained from the study, the K52 genotype in terms of seed yield, the K11 genotype in terms of oil rate and the K52 genotype in terms of protein rate came to the fore.

Keywords: Camelina, Yield ve yield components, Oil composition, Bolu

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INTRODUCTION

Countries have turned to research new and renewable energy sources because of the high share of fossil fuels in energy production and consequently the limited and continuous decrease of fossil fuel reserves and the negative effects of fossil fuels on the environment and human health. For this reason, the tendency to new and renewable energy sources is increasing day by day due to reasons such as diversifying energy sources and reducing their dependence on foreign energy. When alternative energy sources and developments in this field are examined, the development of biofuels such as biodiesel and bioethanol draws attention. The fact that the raw material of biofuels is agricultural products increases the importance of the subject for the agricultural sector and producers (Yaşar and Eren, 2008).

The developments in the vegetable oil and by-products sector have accelerated the agriculture of oilseed plants in the world and in Türkiye in recent years. Obtaining biofuel from vegetable oils has also positively affected the demand for the production of oilseed plants (Kaya and Eryiğit 2020, Yaşar and Sezgin, 2022). Oilseeds take 4th place among our country's top 10 import items (TUIK, 2022). In Türkiye, biofuel blending has been mandated by EPDK (Republic of

Türkiye Energy Market Regulatory Authority), provided that gasoline and diesel fuel are produced from domestic raw materials. In addition, the obligation to blend biodiesel in diesel oil is 0.5%. This obligation means approximately 105,000 tons of biodiesel blending annually. Türkiye is an importer of vegetable oil and has an annual foreign trade deficit of 1.657 billion dollars (TUIK, 2022). To eliminate this negativity, it is necessary to turn to alternative oil sources and increase vegetable oil production. In this sense, the camelina plant is an important alternative.

Camelina (Camelina sativa L. CRANTZ) is also called by different names such as false flax, German sesame, and Siberian oilseed (Kurt and Seyis, 2008). It spreads naturally in the Mediterranean and Central Asia and has been cultivated for 3000 years (Putnam et al., 1993; Zubr, 1997). It was grown economically in Belgium, France, the Netherlands and the Balkans until the 1930s, in Sweden and Poland until the 1950s, and in the Soviet Union until the 1960s (Zubr, 1997). In the following years, it is seen that this plant has taken the place of camelina due to the spread of varieties without erucic acid as a result of breeding studies on rapeseed (Crowley and Fröhlich, 1998). Although the fat content in camelina varies according to the cultivation and climatic factors, it shows a wide variation between 23-48% (Vollmann et al., 2005 and Katar, 2013). The oil rate varies not only according to the cultivation and climatic characteristics but also according to the variety used. Qualified biodiesel by ASTM D6571 and EN14214 biodiesel standards can be obtained from its oil (Wu and Leung, 2011). It is a plant that can be grown without the use of herbicides and is not damaged by the invasion of common parasitic insects due to the low rate of weeds due to its fertilizer demand and allopathic effect in the area where it is grown. In addition, due to its resistance to diseases, the use of chemicals

is low and this makes the plant an environmentally friendly plant. Camelina is relatively drought-resistant and can be grown in very different areas with different climates and soil structures, except in heavy clay and organic soils (Zubr, 1997).

In the vehicle test with the oil obtained from the seed of the plant and the mineral diesel fuel, it was determined that the power of the plant oil (43.25 kW) was higher than the mineral diesel fuel (38.50 kW), and the fuel consumption (12.57 km/l) was less than the mineral diesel fuel (14.03 km/l). It has been determined that smoke turbidity and CO from the exhaust at engine speeds of more than 2000 rpm are approximately 50% lower than that of mineral diesel oil (Bernardo et al., 1998). Fröhlich and Rice (2005) investigated the evaluation of camelina oil as a feedstock for biodiesel production and found that it gave ester yields similar to rapeseed oil and that the iodine value of the camelina oil ester did not meet European Union standards, but other fuel properties were satisfactory. Camelina is primarily preferred among other vegetable oils due to its oil content and high oil yield per hectare, high-quality renewable fuel properties such as jet fuels (cold flow properties, oxidative stability, kinematic viscosity, cetane number, etc.). In addition, it has similar properties to biodiesel prepared from soybean oil, but because the oil contains high levels of linolenic (32.6%), linoleic (19.6%) and oleic (18.6%) acids, methyl and ethyl esters are compared to canola, palm and soybean oil methyl esters. It has lower oxidation stability and higher iodine values (Moser and Vaughn, 2010). At the same time, the fuel properties of camelina oil methyl and ethyl esters such as cold filter plugging point, acid value, cetane number, kinematic viscosity, fluidity, sulfur and phosphorus contents, surface tension are similar to those of canola, palm and soybean oil methyl esters in low sulfur diesel. It has a mixture of components in its fuel. In addition to these studies, Zaleckas et al. (2012) reported that they had positive results in their studies on the biodiesel properties of camelina oil.

Camelina oil is an important source of Omega-3 with its high content of linolenic fatty acid (C18:3). Linoleic fatty acid is one of the best-known polyunsaturated fatty acids. The importance of polyunsaturated fatty acids in human health and nutrition is very high (Broun et al., 1999; Das, 2016; Horrobin, 2000; Mills et al., 2005; Napier and Sayanova, 2005). Many polyunsaturated fatty acids are essential and obtained from diets. Mammals, including humans, cannot synthesize essential fatty acids such as linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). From this point of view, camelina oil and oily pulp can have an important place in human and animal nutrition.

Camelina, which is an annual culture plant, has summer, winter and middle forms. It is a self-pollinating plant. Its fruits are in the form of capsules and contain 15 oval-shaped yellow-yellow brown seeds. 1000 seeds weight varies between 0.8-1.8 g. Seed yield is 2.6 t ha-1 in summer varieties, 3.3 t ha-1 in winter varieties, oil rate of summer varieties is 42%, and winter varieties are 45%. The unsaturated fatty acids in its oil are 90% and approximately 50% of the total fatty acids are composed of polyunsaturated linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). It is reported that the erucic acid (22:1n-9) content in its oil is about 3% and the tocopherol content is about 700 mg kg-1 (Zubr, 1997). It is also reported that it contains many natural antioxidants such as tocopherols, which make the oil stable and usable as edible oil (Kurt and Seyis, 2008).

After removing the oil from the camelina, the remaining pulp contains 10% oil, 45% protein, 13% fibre, 5% mineral substance, and a small amount of vitamins and other substances. The protein of camelina pulp is characterized by the presence of essential amino acids such as arginine, cystine, lysine, methionine and threonine. The composition of amino acids in camelina protein is particularly suitable for feeding poultry (Fogelfors, 1984). It is stated that the pulp of camelina is suitable for feeding ruminant animals and pigs and has high protein and energy content (Schuster and

Friedt, 1998; Koç and Önder, 2012; Matthas and Zubr, 2000).

Camille, a plant that can be grown in marginal areas, is drought tolerant, early maturing and requires less input than other oilseed crops (Urbaniak et al., 2008). In addition, it is supported by the literature that one of the most important alternative oil crops, which will contribute to closing the oil deficit of our country and help meet the raw material needs of the biodiesel industry, is camelina.

One of the most important traits for breeders and researchers is the ability to select genotypes that perform well in a wide variety of environmental conditions (Yaşar, 2023). For this reason, it is aimed to determine the genotypes tdetermine the genotypes that stand out in terms of the traits examined in Bolu ecological conditions.

MATERIALS AND METHODS

In the research, seeds belonging to 8 camelina genotypes, which were prominent in terms of oil rate, seed and oil yield, were used in the trial established in Bolu province, out of a total of 52 genotypes obtained from the seed bank of the United States Department of Agriculture, Agricultural Research Service and Germany (Table 1).

Table 1. Seed materials and origins used in the trial

Trial Code	Original Code	Origin
K-11	PI 304271	Sweden
K-32	PI 650154	Russia
K-36	PI 650158	Poland
K-41	PI 650164	Austria
K-43	PI 650166	Russia
K-45	PI 650168	United States
K-46	PI 652885	Slovenia
K-52	PI 650161	Russia

The field trial was carried out in the research and application field of Bolu Abant İzzet Baysal University, Faculty of Agriculture. Sowing of the trial was done on 1 October (Katar et al., 2012a).

The trial was established in three replications according to the Randomied Complete Block Design, each plot consisted of 6 rows, the plot length was 5 m and the width was 1.2 m. The seeds were sown by hand, with the calculation of 1 kg da^{-1} per decare, equal to 6 rows with 20 cm row spacing (Crowley and Fröhlich, 1998). During the development period of the plants, only weed control was carried out, irrigation and fertilization were not carried out. At the harvest, 0.5 m from the bottom and top of the plot, and one row from the sides were considered as edge effect and the 4 rows in the middle were harvested.

The saturation rate of the soil texture of the trial area is 78.1% and it shows clayey characteristics. Soil pH is 7.48 and shows neutral character. The total amount of salt is 0.02%, it is structurally salt-free and there is no salt problem. The lime value is around 6.39% and it is in a medium calcareous state. It contains moderate organic matter at the level of 2.32% as organic matter. It is very low in available phosphorus (0.06 kg da^{-1}) and high in available potassium (47.7 kg da^{-1}). A total of 637 mm precipitation was observed in the trial area throughout the year, the lowest average temperature value was 0.8 °C in January, and the highest average temperature value was 20.6°C in July and August.

In the study, plant height (cm), number of branches (number $plant^{-1}$), maturation time (day), seed yield per plant (g $plant^{-1}$), 1000 seed weight (g), fixed oil ratio (%), oil yield (kg ha^{-1}), fatty acid composition (%), protein content (%) and protein yield (kg ha^{-1}) were investigated.

The oil content was determined by the solvent (hexane) extraction method in the Soxterm 2000 oil analyzer. In order to determine the fatty acid composition, 10 ml of n-hexane was added to 0.1 g oil, 0.5 ml of 2N methanolic KOH was added, mixed and esterified was ensured by shaking for ½ hour. Shimadzu GC-2010 (Japan), flame ionization detector (FID) and Technochroma Capillary column (100 m x 0.25 mm and 0.2 µm film thickness) were used for determination. Isothermal condition of the column furnace, in which helium was applied as the carrier gas with a flow rate of 0.94 ml/min, Split ratio was set as 1:100, operating temperature was set as 250°C for the injection block and the detector. It is programmed to rise to 240 °C with the temperature increase rate and wait for 20 minutes. In the identification of fatty acids, Restek 35077, Food Industry FAME mix (USA) was used as a standard.

The protein content of homogenous sample taken from camelina seeds obtained from each row was made by Dumas method (Velp Scientifica NDA-701) according to AOAC 992.23: Crude Protein in Cereal Grains and Oilseeds method. In the calculation of the protein, the nitrogen factor was taken as 6.25.

Analysis of variance (ANOVA) was performed with JMP Pro11 (SAS Institute Inc., Cary, NC). The mean values of the properties were compared using the Tukey multiple range test (P > 0.05).

RESULTS AND DISCUSSION

The mean values, the groups formed and the results of the analysis of variance of the values of the properties examined in the experiment are given in Table 2.

Table 2. Mean values, formed groups and variance analysis results of the properties examined in the experiment.

Genotypes	PH	DS	SW	SY	OC	OY	PC	PY
ARSLANBEY	70.50 ± 0.71 a	4.85 ± 0.07 abc	1.19 ± 0.10 ab	1233.4 ± 6.27 bc	38.95 ± 0.92 abc	480.7 ± 3.58 bc	25.70 ± 0.62 abc	317.2 ± 2.38 bcd
K11	66.33 ± 4.04 a	4.53 ± 0.93 abc	1.24 ± 0.06 a	1230.1 ± 2.75 c	40.37 ± 0.64 a	496.6 ± 1.44 b	24.75 ± 1.02 bc	304.3 ± 0.98 bcd
K13	68.00 ± 1.00 a	3.73 ± 0.40 abc	1.17 ± 0.01 ab	1427.8 ± 7.11 a	37.03 ± 1.45 bc	528.9 ± 3.71 ab	23.65 ± 1.13 c	337.2 ± 0.47 bc
K36	68.67 ± 1.15 a	4.30 ± 0.26 abc	1.18 ± 0.01 ab	1099.3 ± 6.26 c	37.17 ± 1.00 bc	408.3 ± 1.92 d	26.14 ± 0.52 ab	287.6 ± 2.21 cd
K41	69.67 ± 3.21 a	3.33 ± 0.47 bc	1.10 ± 0.08 ab	1194.1 ± 3.86 c	35.87 ± 1.07 c	428.0 ± 0.30 cd	27.26 ± 0.76 a	325.7 ± 1.85 bcd
K45	67.00 ± 3.61 a	3.17 ± 0.49 c	1.10 ± 0.05 ab	1401.5 ± 5.67 ab	36.63 ± 0.67 bc	513.2 ± 1.22 ab	25.34 ± 0.43 abc	355.0 ± 1.42 ab
K48	70.33 ± 4.04 a	4.80 ± 0.40 ab	1.12 ± 0.03 ab	1095.4 ± 7.08 c	38.87 ± 0.75 abc	425.4 ± 1.93 cd	25.49 ± 0.73 abc	279.0 ± 1.28 d
K52	71.00 ± 1.73 a	5.07 ± 0.68 a	1.07 ± 0.01 b	1436.6 ± 5.93 a	39.00 ± 1.68 ab	559.8 ± 1.60 a	27.22 ± 0.93 a	391.3 ± 2.91 a
HSD 0.05	-81.7	-15.4	-01.4	-164.3	-31.0	-60.5	-23.1	-51.4
Analysis of variance	ns	**	*	**	**	**	**	**

PH: Plant height (cm), NB: Number of branches (number plant^{-1}), SW: 1000 seed weight, SY: Seed yield (kg ha^{-1}), OC: Oil content (%), OY: Oil yield (kg ha^{-1}), PC: Protein content (%), PY: Protein yield (kg ha^{-1}). HSD: Honestly Significant Difference

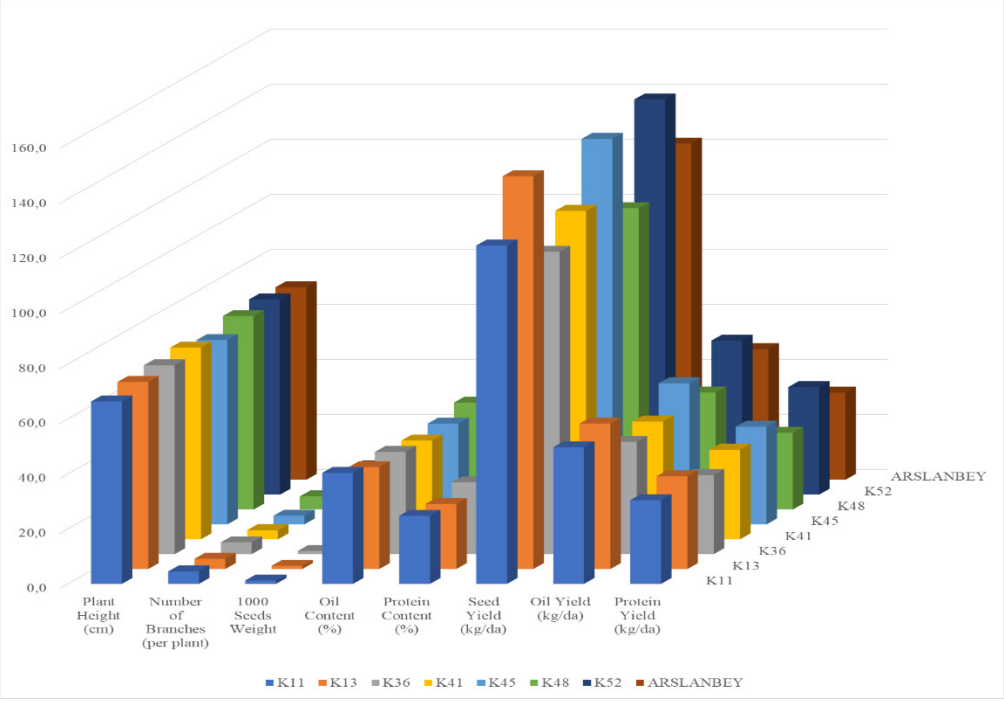

Figure 1. The graph of the mean values of the examined traits

When Table 2, where the analysis of variance values of the trial is given, is examined, it is seen that there is no statistically significant difference between the genotypes in terms of BD, there is a significance at the level of 0.05 in terms of SW, and the difference between the values of NB, SY, OC, OY, PC and PY is significant at the level of 0.001; It is seen that there are no groups in plant height values due to the difference between genotypes, three different groups are formed in NB, two in SW, three in SY, three in OC, three in PC and four in PY.

When the findings were examined, it was observed that in terms of PH, it varied between 66.33 and 71.00 cm and the highest PH value was from the K52 genotype; In terms of NB, it varies between 3.17 and 5.07 and the highest NB value is from the K52 genotype; In terms of SW, it varied between 1.07 and 1.24 g and the highest SW value is from the K11 genotype; In terms of SY, it varies between 1095.4 and 1436.6 kg ha^{-1} and has the highest SY value from K52 and K13 genotypes; The HR values varied between 35.87% and 40.37%, and the highest HR value was from the K11 genotype; OY values varied between 408.3 and 559.8 kg ha^{-1} and the highest OY value was from the K52 genotype; The highest PY value was obtained from the K52 genotype, with the PC values varying between 23.65% and 27.22%, the highest PC value was obtained from the K52 genotype, and the PY values varying between 279.0 and 391.3 kg ha^{-1}. When all the findings are evaluated together, it is seen that the K52 genotype stands out in terms of oil yield and protein yield, which are the main criteria in oilseed plants (Table 2, Figure 1).

Considering the previous studies on camelina, the plant height in camelina was determined by Köse et al. (2018): 72.8-91.5 cm, Francis and Warwick (2009): 30-60 cm, Koncius and Karcauskiene (2010): 50.4-77.9 cm, Çoban and Önder, 2015: 69.0-97.3 cm, Arslan et al. (2014): 116.4-129.7 cm and Arslan et al. (2022): They reported that they detected it in the range of 103.8-59.5 cm. Katar and Katar (2017) reported in their research that the height of the camelina plant was significantly affected by the differences in climatic conditions. Plant height is under pressure from the environment rather than genetics. When these genotypes are tested in a different ecology, they may exhibit different plant heights due to the difference in the response of the genotypes to the environment.

The number of branches in the plant is one of the important factors affecting the yield, especially in oil plants (Vollman and Rajcan, 2009). Considering the results of the research, it is seen that the K52 genotype, which stands out in terms of the number of branches, also stands out in seed yield. The number of branches in the plant, many researchers in their studies, Katar et al. (2012a): 11.45 pieces, Arslan et al. (2014): 3.7-8, and Köse et al. (2018): 8.1-14.7. Although branching is a genetic trait, it increases or decreases depending on the planting frequency. The same genotype may show more branching tendency in sparse planting compared to dense planting. In addition, due to adverse environmental conditions, especially drought and insufficient nitrogen amount, the plant cannot grow as much as its genetic capacity requires and can form fewer branches.

Another important yield factor in camelina plant is 1000 seed weight (Vollman and Rajcan, 2009). This trait is high; It is important in terms of extracting oil from the seed more effectively and providing faster and stronger output in adverse climate and soil conditions (Qatar and Qatar, 2017). It is reported that the 1000 seed weight is a genetic trait as well as being affected by environmental conditions (Köse et al., 2018; Akk and Ilumae, 2005). Regarding the 1000 seed weight in previous studies, Mason (2009): 1.19 g, Katar et al., (2012a): 0.44 g, Katar et al. 2012b: 1.24 g and Katar et al. (2012c): 1.16 g, Arslan et al. (2014): 1.18-1.31 g, Agegnehu (1997): 1.45 g, Köse et al. (2018): 1.04-1.28 g and Arslan et al. (2022): They reported that they detected it in the range of 1.50-0.84 g. The values related to 1000 seed weight obtained from this study show similarities and differences with previous studies. The fact that the 1000 seed weight is a genetic trait as well as being affected by environmental conditions makes the differences understandable.

The main purpose in the production of oil crops is to increase seed and oil yield. Two important factors affecting oil yield in camelina plant are seed yield and oil content (Katar et al. 2012a). It has been reported that the seed yield, oil rate and, accordingly, the oil yield are largely under the influence of environmental conditions, and the performances of genotypes differ according to changing environmental conditions (Seehuber, 1984; Koncius and Karcauskiene, 2010). Regarding the seed yield in the studies, Katar et al. (2012): 48.2-700.0 kg ha^{-1}, Arslan et al. (2014): 875.3-1811.3 kg ha^{-1}, Köse et al. (2018): 478-1024 kg ha^{-1} and Arslan et al. (2022), They reported that they detected it in the range of 188-3152 kg ha^{-1}. Seed yield in oilseed plants is a trait that is highly affected by environmental conditions as well as genetic capacity. Therefore, the same genotype can exhibit very different yield potential in different ecologies or conditions. This study reveals the performance of genotypes in Bolu ecological conditions in terms of seed yield.

In the breeding program of oilseed plants, oil content is one of the main breeding criteria. The oil content trait determined by the genetic structure can be listed with the effect of environmental conditions. The plant can reveal its existing genetic capacity only if there are optimum conditions. Therefore, the oil content in the seed may fall below the genetic capacity depending on the environment and growing conditions. Arslan et al., (2014) reported that phosphorus and nitrogen applications affect the oil content in camelina positively, and nitrogen and phosphorus applications increase it up to a certain dose. Regarding seed yield in previous studies, Katar et al. (2012a): 21.4-35.4%, Katar et al. (2012d): 23.4-32.7%, Köse et al. (2018): 30.0-34.3 %, Subasi et al. (2021): 34.35-37.88% and Subaşı et al.

(2022): They reported that they found it in the range of 26.69-39.17%.

After the oil is removed from the seeds of the oilseed plants, the remaining meal is a very valuable animal feed because of the protein they contain. The main criterion that determines the feed value of the pulp is the high or low digestible protein content. For this reason, it is desirable that the protein content in the seed is high as well as the oil content. Protein content, like oil content, is one of the features controlled by genetic structure, and it reveals the plant's capacity in optimum environmental conditions, otherwise, it cannot display its genetic potential. Therefore, the environment and cultivation techniques can affect the protein content. The same genotypes can give different protein content values in different ecologies or environmental conditions. Regarding protein content in previous studies, Subaşı et al. (2021): Reported that they detected it in the range of 25.76%-27.64%.

There were statistically significant differences between genotypes in terms of oil components. While there was no difference between the genotypes in terms of C22:2, C24:0 and C24:1 fatty acids, C20:0 fatty acids were at the level of 0.05, C16:0, C18:0, C18:1, C18:2, C18:3, C20:2. The difference between C22:0 and C22:1 fatty acids was 0.01 (Table 3).

Table 3. Mean values of the oil components of the genotypes, the resulting groups and variance analysis results.

Level	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:2	C22:0	C22:1	C22:2	C24:0	C24:1
ARSLANBEY	5.26 ± 0.05 ab	2.70 ± 0.00 ab	15.86 ± 0.07 ab	17.09 ± 0.02 c	50.14 ± 0.10 bc	1.30 ± 0.17 ab	1.83 ± 0.00 bc	0.31 ± 0.00 a	4.28 ± 0.00 a	0.16 ± 0.01 a	0.17 ± 0.00 a	0.90 ± 0.00 ab
K11	5.41 ± 0.07 a	2.65 ± 0.01 ab	16.13 ± 0.09 a	17.50 ± 0.09 bc	49.43 ± 0.36 c	1.35 ± 0.11 ab	1.83 ± 0.01 bc	0.27 ± 0.00 bc	4.16 ± 0.02 ab	0.15 ± 0.00 a	0.17 ± 0.00 a	0.84 ± 0.00 ab
K13	5.49 ± 0.09 a	2.58 ± 0.11 bc	13.88 ± 0.15 c	17.45 ± 0.34 bc	51.65 ± 0.72 a	1.49 ± 0.01 a	1.98 ± 0.01 a	0.31 ± 0.01 a	3.94 ± 0.48 abc	0.18 ± 0.02 a	0.16 ± 0.01 a	0.86 ± 0.25 ab
K36	5.35 ± 0.08 ab	2.74 ± 0.01 a	15.97 ± 0.27 ab	17.54 ± 0.25 bc	49.60 ± 0.16 bc	1.45 ± 0.02 ab	1.83 ± 0.03 bc	0.31 ± 0.01 a	4.04 ± 0.17 abc	0.16 ± 0.00 a	0.16 ± 0.01 a	0.82 ± 0.08 ab
K41	5.32 ± 0.05 ab	2.71 ± 0.09 ab	15.96 ± 0.34 ab	17.41 ± 0.17 bc	50.68 ± 0.55 ab	1.33 ± 0.01 ab	1.75 ± 0.06 c	0.28 ± 0.01 bc	3.53 ± 0.26 bc	0.15 ± 0.01 a	0.32 ± 0.29 a	0.78 ± 0.05 ab
K45	5.49 ± 0.02 a	2.48 ± 0.01 c	14.44 ± 0.23 c	17.13 ± 0.12 c	51.62 ± 0.35 a	1.36 ± 0.01 ab	1.96 ± 0.02 a	0.29 ± 0.02 abc	3.98 ± 0.13 abc	0.18 ± 0.01 a	0.14 ± 0.01 a	0.86 ± 0.02 ab
K48	5.14 ± 0.10 b	2.67 ± 0.04 ab	15.52 ± 0.19 b	17.81 ± 0.18 ab	49.90 ± 0.28 bc	1.43 ± 0.04 ab	1.86 ± 0.02 b	0.30 ± 0.01 ab	4.02 ± 0.12 abc	0.17 ± 0.01 a	0.17 ± 0.00 a	0.95 ± 0.02 a
K52	5.44 ± 0.15 a	2.64 ± 0.01 ab	16.06 ± 0.19 ab	18.30 ± 0.31 a	49.71 ± 0.20 bc	1.28 ± 0.00 b	1.84 ± 0.04 b	0.27 ± 0.01 c	3.51 ± 0.22 c	0.15 ± 0.02 a	0.32 ± 0.26 a	0.65 ± 0.05 b
HSD0.05	0.24	0.15	0.59	0.60	0.21	1.11	0.08	0.03	0.64	0.03	0.39	0.27
Variance Analysis	**	**	**	**	**	*	**	**	**	ns	ns	ns

In previous studies on the plant; Katar et al. (2012d) C16:0 in the range 6.21-6.82%, C18:0 in the range of 2.43-2.90%, C18:1 in the range of 15.1-17.1%, C18:2 in the range of 18.6-2.4%, C18 they found :3 in the range of 30.5-33.4%, C20:0 in the range of 1.6-2.0%, C20:2 in the range of 1.7-1.9%, and C22:1 in the range of 2.9-3.6%; Qatar et al. (2012a) C16:0 in the range of 5.9-7.1%, C18:0 in the range of 2.5-3.0%, C18:1 in the range of 16.0-17.6%, C18:2 in the range of 18.5-23.4% , C18:3 in the range of 24.9-32.3%, C20:0 in the range of 0.3-1.9%, C20:2 in the range of 1.3-1.9%, C22:0 in the range of 0.3-1.9, and C22:1 in the range of 2.9 They found in the range of -3.5%; Arslan et al. (2014) C16:0 in the range 5.1-6.1%, C18:0 in the range of 2.6-2.9%, C18:1 in the range of 15.9-19.8%, C18:2 in the range of 17.1-21.5%, C18 they found:3 in the range of 26.3-34.1%, C20:0 in the range of 1.4-1.8%, and C22:1 in the range of 2.56-3.72%; Kıralan et al. (2018) C18:1 in the range of 5.1-5.2%, C18:2 in the range of 17.1-17.7%, C20:0 in the range of 1.5-1.6%, C18:3 in the range of 33.9-34.6%, and C22, They found:1 in the range of 2.80-2.82 and Subaşı et al. (2022) reported that they found C18:3 in the range of 8-36% and C22:1 in the range of 2.1-5.0%. When the values obtained from studies conducted with different genotypes in different years and ecological conditions are examined, the fatty acid ratio values obtained from this study show similarities with the exception of the linolenic fatty acid ratio. Looking at the data obtained from existing studies, it can be said that linolenic fatty acid ratios differ more than other fatty acid ratios and this fatty acid is more affected by environmental conditions.

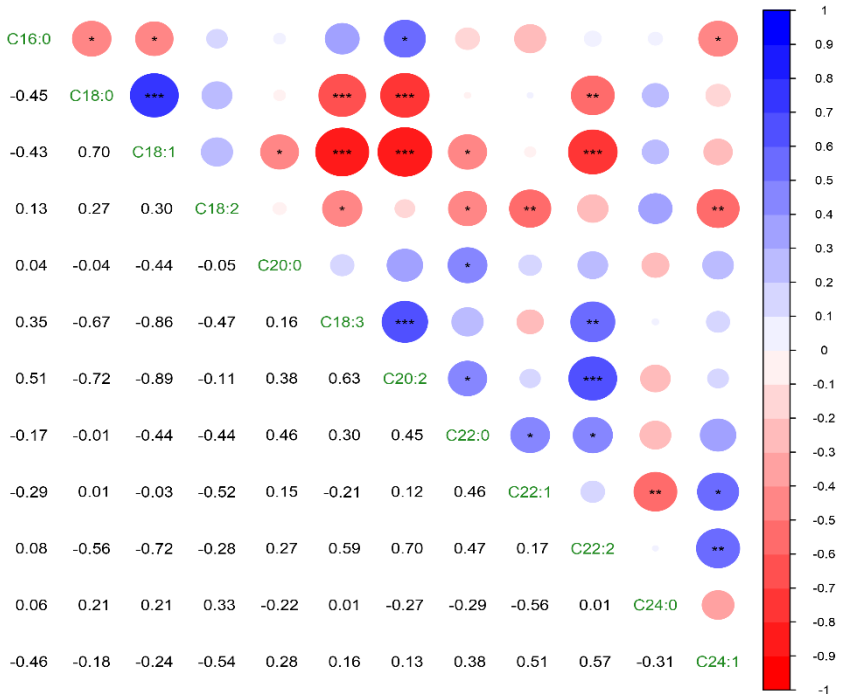

Figure 2. Fatty acids correlation chart

When the findings obtained from this study are examined, it is seen that between C16:0 and C18:1 and C24:1, between C18:1 and C20:0, C18:3, C20:2 and C22:1, between C18:2 and C18:3 and C20. :0 and C22:1 to C24:0 are negative; It is seen that there is a positive correlation between C16:0 and C20:2, between C20:0 and C22:0, between C18: and C20:2, between C20:2 and C22:0 and C22:2, and between C22:0 and C22:1 (Fig. 2).

Although there are not many studies showing the correlation between the fatty acid components of the camelina plant, Subaşı et al. (2022), in their study with 39 camelina genotypes, there was a negative correlation between C18:1 and C18:2 and C18:3, and between C18:2 and C18:3 and C16:0; It is reported that there is a positive correlation between C18:1 and C16:0 and between C18:2 and C18:3.

CONCLUSION

In the study, no great variation was observed among the camelina genotypes in terms of the major fatty acids palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). Considering that K13 and K52 genotypes may be suitable genotypes in terms of seed yield among the camelina genotypes, but K52 genotype is superior to other genotypes in terms of oil yield and protein yield, it has been determined that it is the most suitable genotype for Bolu ecological conditions and similar climatic and soil conditions.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

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RESEARCH ARTICLE

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How growing conditions are influential on the agronomic attributes and fiber-related quality parameters of flax (*Linum usitatissimum* L.) fibers: A seismomorphogenesis approach

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Abstract

Among industrial crops, flax (*Linum usitatissimum* L.) is a multi-purpose crop grown for production of stem fiber and seed oil. Through longterm domestication for these purposes, cultivated flax has diversified into two main types, namely fiber and oil or linseed types, as well as an intermediate type. This study was designed to investigate the effect of flax fibers of flax varieties (Antares, Dakota and Mures) in field and greenhouse cultivation on fiber mechanical performances, morphological properties and fatty acid composition in flaxseed was investigated. Additionally, plants can change their morphology and mechanical properties when exposed to stress, as is particularly the case when plants respond to wind, a phenomenon known as seismomorphogenesis. Greenhouse plants were found to be significantly lower than field grown plants, with an increase of 16.79cm in technical stem length and 56.02cm in technical stem height. In addition, the total plant height of flax grown in the field was 59.33 cm compared to 17.32 cm in the greenhouse. The Mures variety was found to be the highest both in the field (79.50-76.10cm) and in the greenhouse (18.36-17.47cm). Considering the fatty acid percentages, the highest α-linolenic acid was found in Dakota (29.23%) and the lowest in Antares (20.53%) in the field, while the highest was found in Dakota (19.25%) and the lowest in Mures (16.13%) in the greenhouse. In addition, the highest tensile strength was found in Dakota (704.6 Mpa) and the closest Antares (692.2 Mpa) under field conditions, while the lowest was found in Dakota (198.5 Mpa) and Mures varieties (288.8 Mpa) under greenhouse conditions. In order to reduce the dimension, correlate and visualize the measured parameters, the relevant data of the study were subjected to principal component analysis and heat-map clustering.

Keywords: Flaxseed, Linum usitatissimum, Plant muscles, Seismomorphogenesis, SEM

INTRODUCTION

Flax/Linseed (*Linum usitatissimum* L.), one of the oldest plants grown for its seeds or fiber s among industrial crops, is grown as; i) oil flax for flaxseed oil production and ii) fiber flax for fiber production (Saleem et al., 2020; Koçak, 2022; Stavropoulos et al., 2023). In addition, the genus Linum, belonging to the Linaceae family is an herbaceous, annual, self-pollinated, oldest domesticated diploid species (Sarmast et al., 2014; Tork et al., 2019). Flax can be called by many different names. In Türkiye, local names such as "bezir, bızıktan, cimit, kön, siyelek and zeyrek"are used for the flax plant (Davis, 1982; Dumanoğlu, 2020). There is evidence for it being used in ancient Egypt and Mesopotamia (Carmona and Ezzamel, 2007), with Ethiopia, Central Asia, and India being considered to be

secondary centers. The species is distributed in other regions of the world as well, including the Middle East, North and Southwest America, and the Mediterranean Basin (Vavilov 1951; Chen 2022). Its also an important cultivated plant grown for its fiber and oil-rich seeds (Choudhary et al., 2017; Saroha et al., 2022). Mechanical conditioning is the deliberate application of physical stimulation and stress to control the growth and quality of plants (Koçak et al., 2023). Flax fiber has always been used for textile production. In addition to clothing and upholstery, a newer tech-nical application involving the use of flax fibers as reinforcements for composite materials has been extensively developed over the last few decades (Goutianos et al., 2006; Yan et al., 2014). Besides, flax fibers play an important role as reinforcement by supporting plant tissues in the stem and can even act as "plant muscles" (Gorshkova et al., 2012; Baley et al., 2018). Due to its industrial importance, flax has been subjected to a century of extensive variety selection aimed at increasing fiber yield and the plants' lodging stability and resistance to diseases (Bourmaud et al., 2016; Goudenhooft, 2017). One of the main concerns is the improvement of fiber mechanical properties of the fibers optimise the mechanical performance of composites. In addition, results from the literature show that although variety selection can increase fiber yield, fiber mechanical properties are less affected by flax variety (Van de Weyenberg, 2003; Omrani et al., 2017). The effect of mechanically induced stress (MIS) on plant traits such as yield or quality has also been studied. As well as being a natural source caused by wind, rain or animal movement, MIS can also be induced in greenhouses by rubbing or brushing plants. The plant response is called 'tigmomorphogenesis' in the case of physical contact and 'seismomorphogenesis' in the case of wind action (Börnke and Rocksch, 2018; Goudenhooft et al., 2018; Šic Žlabur et al., 2021). Additionally, the first example of such responses is the reduction in plant height that usually occurs in plants exposed to wind or other MIS (Biddington, 1986). In this context, it has been shown that trees exposed to wind are shorter and more pointed, but have lower overall stiffness, although their mechanical properties are less affected by their morphology (Paul-Victor and Rowe, 2011; Shiba et al., 2023). In contrast, wheat plants are stronger and less flexible, but do not show a significant change in stem height under the influence of wind sway (Crook and Ennos, 1996). Seismomorphogenesis causes changes in plant characteristics such as stem diameter, fiber quality or chlorophyll content, with different responses to observations made between species. In addition to causing morphological and mechanical changes in plants, seismomorphogenesis has generally been reported to result in a significant increase in crop yield when plants are grown in the greenhouse under optimum conditions (Koçak et al., 2023). Flax is an industrial crop usually grown in the field and changes in morphology and fiber yield are expected when grown under much milder windy conditions. From this point of view, it is of great interest to grow flax in a greenhouse environment and to study fiber yields (Heller et al., 2015; Jacobsson, 2018). Flax, like any plant, responds to external stresses and its development and stem structure can be affected by seismomorphogenesis, gravitropizm and growing conditions (Moulia et al., 2016).

Concerning industrial uses of the crop, the beneficial effects are mainly due to the lipids in flaxseed. Flaxseed oil is one of the richest plant sources of α-linolenic acid (omega-3) and linoleic acid (omega-6) polyunsaturated fatty acids (PUFAs), which are essential for human nutrition as they cannot be synthesized by the body. In addition to other important uses, flaxseed is characterized by its oil content of approximately 30-60%. The oils in flaxseed contain valuable amounts of the fatty acid compounds α-linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid', as well as high levels of lignans, fiber, protein, vitamins and micronutrients (Dubey et al., 2020; Deme et al., 2021; Yang et al., 2021). Corresponding to the litarature review/potential mechanism of the plants, it was hypothesized that plants grown under openfield conditions would be thicker and dense structure. This study investigates the effect of seismomorphogenesis on the stems and fibres of registered flax cultivars under greenhouse and field conditions in terms of structure/mechanical properties and quality. It also includes a comparison between field-grown and greenhouse grown flax at plant maturity. This comparison is firstly based on morphological analysis of flax stalks and fibre structure properties. Subsequently, changes in the quality (fatty acid composition) of flax seeds were evaluated.

MATERIALS AND METHODS

Plant material and experimental design

The relevant study was conducted at the greenhouses (grown in greenhouses for a 14/10 h photoperiod, 26–30 °C/ day and 16–20 °C/ night; relative humidity: 60%) and experimental area in 2022 at the Agricultural Application and Research Center of Iğdır University using in a randomized block design with three replications. The flaxseeds were provided by different agricultural institutions in Türkiye. The flaxseeds of the foreign registered varieties (Antares, Dakota and Mures) was obtained from different provinces of Türkiye. Agromorphological traits were determined 84 days after sowing by randomly selecting 5 plants from each plot. In this context, promising flaxseed varieties for oil and natural fiber have been identified.

Experimental area and characteristics of the experimental soil

The Iğdır region is the lowest plain of the East Anatolian region with microclimatic characteristics, and it is one of the largest plains in terms of surface area compared to the surrounding provinces. Due to the microclimatic characteristics

of the Iğdır region; with salty and calcareous soils, not all of its lands are suitable for agriculture. The soil properties used in the field experiment and in the greenhouse environment were determined (Table 1) (Karaoğlu and Çelim, 2018). In addition, the soil used in the greenhouse was taken from the soil used in the field (Kocak et al., 2023).

Table 1. Soil characteristics of the experimental area

Examined trials/analysis types	Value/result	Soil structure
pH	9.70	Strong alkaline
EC (dS/m)	3.05	Moderately tolerant
Total salt (%)	82.44	Too salty
$CaCO_3$ (%)	9.29	Medium lime
Organic matter (%)	0.42	Very little
P_2O_5 (ppm)	0.44	Very little
K_2O (ppm)	42.56	Sufficient
Saturation (%)	52.29	Clay-loamy

Phenotypic evaluation of flax

On five randomly selected plants, 12 morphological plant traits were determined at 84 days after sowing (DAS) from field and greenhouse conditions were determined for fiber and seed traits, respectively; plant height (cm/plant) (PH), number of flowering days (days) (NFD), ripening days (days) (RD), number of siblings (number/plant) (NS), capsule branches per plant (number/plant) (CBPP), capsule branches in plant (number) (CBP), capsule weight per plant (g/plant) (CWPP), number of seeds per plant (number/plant) (NSPP), number of seeds in capsule (number/capsule) (NSC), seed yield per plant (g/plant) (SYPP), stem weight (g/plant) (SW), technical stem length (cm) (TSL) Table 2. Fiber quality parameters were evaluated according to (Pisupati et al., 2021). The variable numbers, codes of each individual morphological trait are presented in Table 3.

Table 2. Morphological characteristics assessed in flax

Trait name	Code	Detail
Plant height(cm)	PH	Height of main stem from cotyledon scar to top boll
Number of flowering days	NFD	The date on which 75% of the plants on the parcel flowered
Ripening days	RD	The period until the maturity of 90% of the plants in the parcel
Number of tillering (number/plant)	NS	Count of the branches
Capsule of branches per plant (number/plant)	CBPP	Primary branches on the plant body
Capsule branches in plant (number)	CBP	Capsules being on each plant
Capsule weight per plant (g/plant)	CWPP	Wight of capsules on each plant
Number of seeds per plant (number/plant)	NSPP	Seeds in capsule per plant
Number of seeds in capsule (number/capsule)	NSC	Seeds in capsule
Seed yield per plant (g/plant)	SYPP	Seeds of plants taken from each parcel
Stem weight (g/plant)	SW	Weight at stem of plants taken from each parcel
Technical stem length (cm)	TSL	From the first cotyledon leaves to the first branching

Extraction of flaxseed oil

Flaxseeds from the field and greenhouse were pulverised and 5g of the sample was treated by Soxhlet extraction with N-hexane for 5 hours. The used seed pulp was then filtered and the solvent was removed in a rotary evaporator (HEIDOLPH Hei-VAP Core (HL/ML)) under vacuum at 40°C. The oil samples were then stored in a refrigerator at 4°C for analysis. The oil obtained after the treatments was considered as the total oil content of the flaxseed (Lamani et al., 2021)

Flaxseed oil yields (kg/ha) were calculated using the following formula;

Flaxseed oil yield (kg/ha) = Oil content (%) x Flaxseed yield (kg/ha) (Xie et al., 2020).

Fatty acid profile using High Performance Liquid Chromatography (GC-FID)

Flaxseed oil (0.2g) was taken into 15 ml centrifuge tubes and shaken with 10 ml of hexane. The samples were dissolved in 0.2mL of 1 N-methanol and KOH was added. The tube was shaken and phase separation was observed and it was

kept in the dark for 2 hours until the upper phase became clear. Clarification after process, some of the upper phase was taken into vials, and fatty acids were analyzed with the help of Agilent 7820 A GC-FID (Agilent Technologies, USA) device with a SP 2560 100m*0.25mm*0.2µm capillary column with a flame ionization detector (FID). Injection port and FID temperature is 240ºC, 1/10 split ratio at 400 ml/min pressure in split injection mode. After waiting for 5 minutes at 140ºC, the column temperature increased by 4ºC per minute to reach 250ºC and reached 260ºC after waiting for 15 minutes. Helium carrier gas 41 cm/sec (Hydrogen) was used. Samples injected with 1 µL into the device were compared with the GC-FID chromatogram obtained in the analysis of the "Supelco ® 37 Component FAME Mix-Sigma-Aldrich" standard mixture for a total of 37.75 minutes. As a result of the analysis; α-linolenic acid (C18:3n6), linoleic acid (C18:2n6), oleic acid (C18:1n9c), Palmitic acid (C16:0), stearic acid (C18:0) fatty acids were determined as % (Table 4).

Mechanical structure of flax fiber and Scanning Electron Microscopy (SEM)

A testing machine with a 5 kN load cell (Zwick/Roell) was used to determine fiber parameters and yield analysis of high fiber flax varieties harvested in the field and in the greenhouse. Diameter measurements (left, right and centre) were made using a digital caliper (MİTUTOYO). Furthermore, images of fibers structures were obtained using scanning electron microscope (SEM) (ZEISS Sigma 300). For the SEM, a field emission scanning electron microscope was used with an accelerating voltage of 10 kV was used to observe the morphology of the leaf samples. Prior to FE-SEM analysis, as herbal products are not electrically conductive, the surface of the samples was coated with a gold plating device, Qurom, to ensure electron scattering from the surface. A secondary electron (SE) detector was used to display the morphological information of the samples.

Data analysis

For the study, three replicates (each with 5 plants) were used and each replicate corresponded to the five seedlings. In order to evaluate the results, the flax plants were compared for their agronomic and morphological attributes using one-way variance analysis ($p<0.05$) (SPSS 22). Pearson correlation (r) was used to determine the association between the investigated characteristics. A principal component analysis (PCA) at (PAST Software) and a heat-map clustering (ClustVis) were employed to define the dependent variable parameters corresponding to the registered varieties (independent variables).

RESULTS

Morphological evaluation

Growth and development parameters were analysed to evaluate the effects of seismomorphogenesis on flax (L. usitatissimum L.) under field and greenhouse conditions. Some basic parameters such as plant height, number of days to flowering, number of days to maturity, number of tillers, capsule branches per plant, capsule weight per plant, number of seeds per plant, number of seeds per capsule, seed yield per plant, stem weight, technical stem length were estimated for the morphological characteristics of the 3 varieties considered for the analysis (Table 3). Greenhouse plants were found to be significantly lower than field-grown plants, with an increase of 16.79cm in technical stem length and 56.02cm in technical stem length. In addition, the total plant height of flax grown in the field was 59.33cm, while the plant height in the greenhouse environment was 17.32cm. Significant differences were found in plant height, number of siblings, capsule branches per plant, number of seeds per plant stem weight and technical stem length between the morphological characteristics of the cultivars (Antares, Dakota, Mures) under field and greenhouse conditions. In terms of plant height and technical stem length among the morphological characteristics between the varieties; the Mures variety was found to be the highest both in the field (79.50-76.10*cm*) and in the greenhouse (18.36-17.47*cm*) (Table 3).

Heat-map clustering and Principal Component Analysis (PCA) of plant growth and development traits

We performed heat-map clustering (Fig. 1) and PCA (Fig. 2) to visualise and clarify the morphological traits associated with cultivars used under field and greenhouse conditions. The present results showed that heat map clustering revealed two main clusters under both growing conditions. While the first cluster was number of flowering days, ripening days in field conditions, number of flowering days, number of seeds in capsule, stem weight in greenhouse conditions, the second main cluster was plant height, number of siblings, capsule branches per plant, capsule branches in plant, capsule weight per plant, number of seeds per plant, number of seeds per plant, number of seeds in capsule, seed yield per plant, stem weight, technical stem length in the field; plant height, ripening days, number of siblings, capsule branches per plant, capsule branches in plant, capsule weight per plant, number of seeds per plant, seed yield per plant, technical stem length were associated with the growth and development characteristics of flax. In order to explain the percentage of variation, PCA was carried out to reveal the kind of relationship and the level of variations between varieties and related parameters (Fig. 2). Accordingly, two components with eigen values above

1 were observed under field conditions. These two components (F_1: 87.7% and F_2: 12.2%) explain a total variation of 99.9%. In addition, two components with eigen values above 1 were observed under greenhouse conditions. These two components (F_1: 57.7% and F_2: 42.1%) explain a total variation of 99.8%.

Table 3. Growth and development parameters of flax varieties used for traits in the field (A) and in the greenhouse (B)

Field morphology(A)

Variety	Plant height(cm/ plant)	Number of flowering days (days)	Ripening days (days)	Number of siblings (number/ plant)	Capsule branches per plant (number/ plant)	Capsule branches in plant (number)	Capsule weight per plant (g/ plant)	Number of seeds per plant (number/ plant)	Number of seeds in capsule (number/ capsule)	Seed yield per plant (g/ plant)	Stem weight (g/ plant)	Technical stem length (cm)
Antares	43.50±0.50c	83.00±3.60a	92.00±2.64a	9.00±1.00b	7.66±0.57a	8.33±0.57b	0.50±0.02b	68.33±3.51c	8.33±0.57b	0.53±0.01b	0.85±0.01c	39.60±1.40c
Dakota	55.00±1.00b	73.33±2.08b	78.66±2.51b	11.33±0.57a	8.66±1.52a	11.00±1.00a	0.55±0.05b	87.33±1.52b	8.33±0.57b	0.56±0.05b	0.92±0.02b	52.36±0.80b
Mures	79.50±0.50	65.50±0.50c	71.00±1.00c	11.00±1.00a	9.33±1.52a	10.66±0.57a	0.71±0.01a	113.33±7.09a	9.66±0.57a	0.69±0.01a	2.11±0.01a	76.10±1.01a
Mean	59.33±15.93a	73.94±7.87	80.55±9.39	10.44±1.33	8.55±1.33	10.00±1.41	0.58±0.10	89.66±19.97	8.77±0.83	0.59±0.08	1.29±0.61	56.02±16.06
F	2028.50	39.332	70.90	6.143	1.267	11.400	26.355	70.662	5.333	19.493	5364.428	847.511
p	.000	.000	.000	.035	.348	.009	.001	.000	.047	.002	.000	.000

Field morphology(A) T test

Variety	Plant height(cm/ plant)		Number of flowering days (days)		Ripening days (days)		Number of siblings (number/ plant)		Capsule branches per plant (number/ plant)		Capsule branches in plant (number)		Capsule weight per plant (g/ plant)		Number of seeds per plant (number/ plant)		Number of seeds in capsule (number/ capsule)		Seed yield per plant (g/plant)		Stem weight (g/plant)		Technical stem length (cm)	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p
Antares* Dakota	-17.816	.000	4.022	.016	6.325	.003	-3.500	.025	-1.061	.349	-4.000	.016	-1.264	.275	-8.593	.001	.000	1.000	-.925	.407	-4.890	.008	-13.679	.000
Antares* Mures	-88.182	.000	8.327	.001	12.860	.000	-2.449	.070	-1.768	.152	-4.950	.008	-14.469	.000	-9.846	.001	-2.828	.047	-13.065	.000	-133.830	.000	-36.561	.000
Dakota* Mures	-37.955	.000	6.337	.003	4.904	.008	.500	.643	-.535	.621	.500	.643	-4.670	.010	-6.205	.003	-2.828	.047	-4.014	.016	-76.122	.000	-31.684	.000

Greenhouse (B)

Variety	Plant height(cm/ plant)	Number of flowering days (days)	Ripening days (days)	Number of siblings (number/ plant)	Capsule branches per plant (number/ plant)	Capsule branches in plant (number)	Capsule weight per plant (g/ plant)	Number of seeds per plant (number/ plant)	Number of seeds in capsule (number/ capsule)	Seed yield per plant (g/ plant)	Stem weight (g/ plant)	Technical stem length (cm)
Antares	16.13±.80b	59.67±2.52a	63.33±0.58a	2.67±0.58a	2.33±0.58a	2.67±0.58a	0.24±0.02b	22.33±5.03a	8.67±0.58a	0.18±0.01b	0.24±0.01a	15.40±0.60b
Dakota	17.46±.50ab	58.00±3.00a	61.00±3.00ab	3.33±0.58a	2.67±0.58a	3.00±1.00a	0.34±0.05a	24.67±7.02a	8.33±0.58a	0.31±0.01a	0.16±0.00b	17.50±0.50a
Mures	18.36±1.00a	55.00±1.00a	58.67±1.53b	2.33±0.58a	2.33±0.58a	3.00±0.00a	0.25±0.01b	24.67±2.08a	8.33±0.58a	0.17±0.01b	0.27±0.02a	17.47±0.47a
Mean	17.32± 1.19	57.56±2.88	61.00±2.65	2.78±0.67	2.44±0.53	2.89±0.60	0.28±0.05	23.89±4.59	8.44±0.53	0.22±0.07	0.22±0.05	16.79±1.14
F	5.949	3.082	4.200	2.333	.333	.250	8.518	.207	.333	181.980	64.927	15.628
p	.038	.120	.072	.178	.729	.787	.018	.819	.729	.000	.000	.004

Greenhouse(B) T test

Variety	Plant height(cm/ plant)		Number of flowering days (days)		Ripening days (days)		Number of siblings (number/ plant)		Capsule branches per plant (number/ plant)		Capsule branches in plant (number)		Capsule weight per plant (g/ plant)		Number of seeds per plant (number/ plant)		Number of seeds in capsule (number/ capsule)		Seed yield per plant (g/ plant)		Stem weight (g/plant)		Technical stem length (cm)	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p
Antares* Dakota	-2.425	.072	.737	.502	1.323	.256	-1.414	.230	-.707	.519	-.500	.643	-3.147	.035	-.468	.664	.707	.519	-16.142	.000	14.059	.000	-4.657	.010
Antares* Mures	-3.005	.040	2.985	.041	4.950	.008	.707	.519	.000	1.000	-1.000	.374	-.875	.431	-.742	.499	.707	.519	1.209	.293	-1.965	.121	-4.687	.009
Dakota* Mures	-1.391	.237	1.643	.176	1.200	.296	2.121	.101	.707	.519	.000	1.000	3.027	.039	.000	1.000	.000	1.000	17.029	.000	-9.915	.001	.084	.937

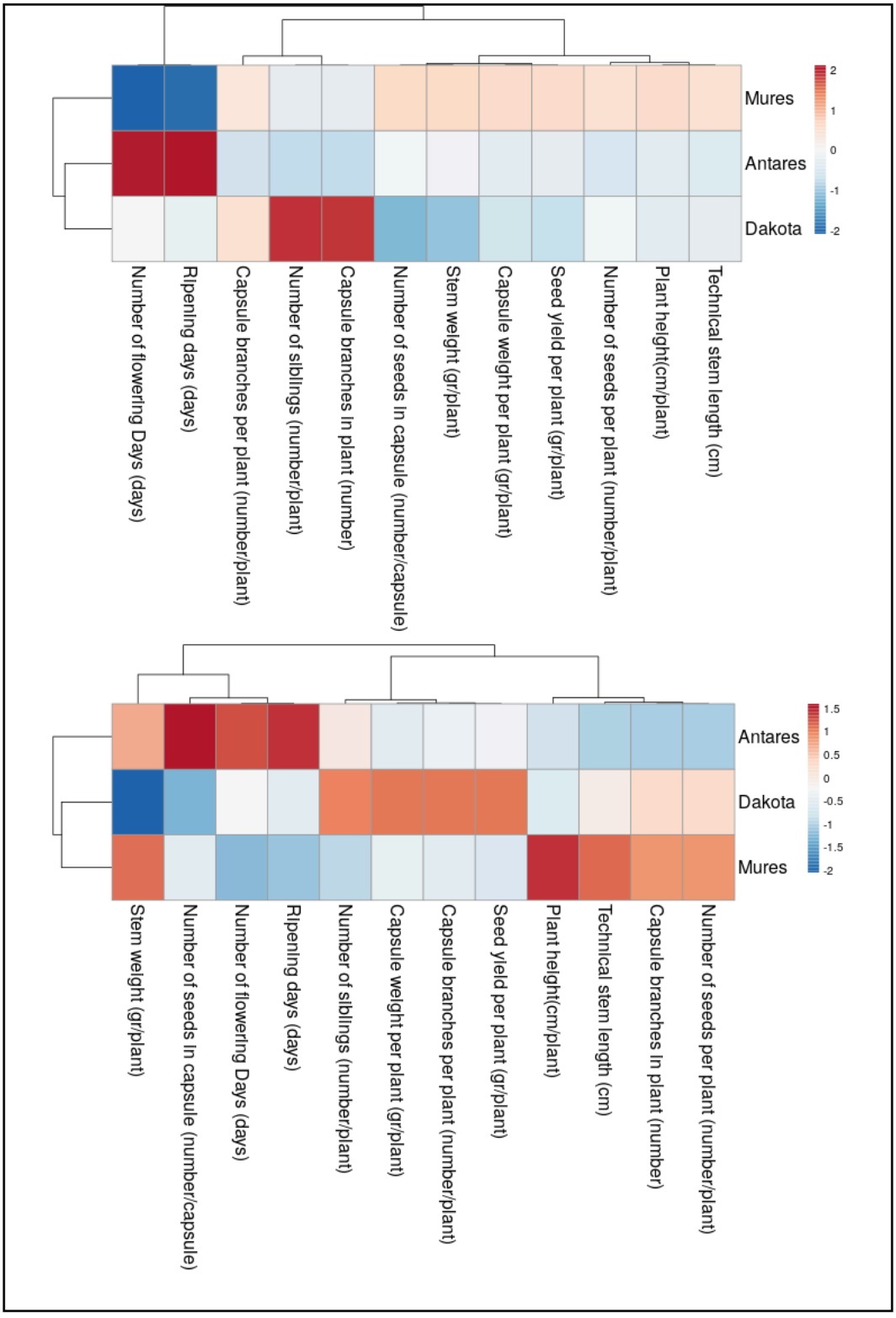

Figure 1. Heatmap clustering of growth and development parameters of flax cultivars used for treatments under the field (A) and the greenhouse conditions (B).

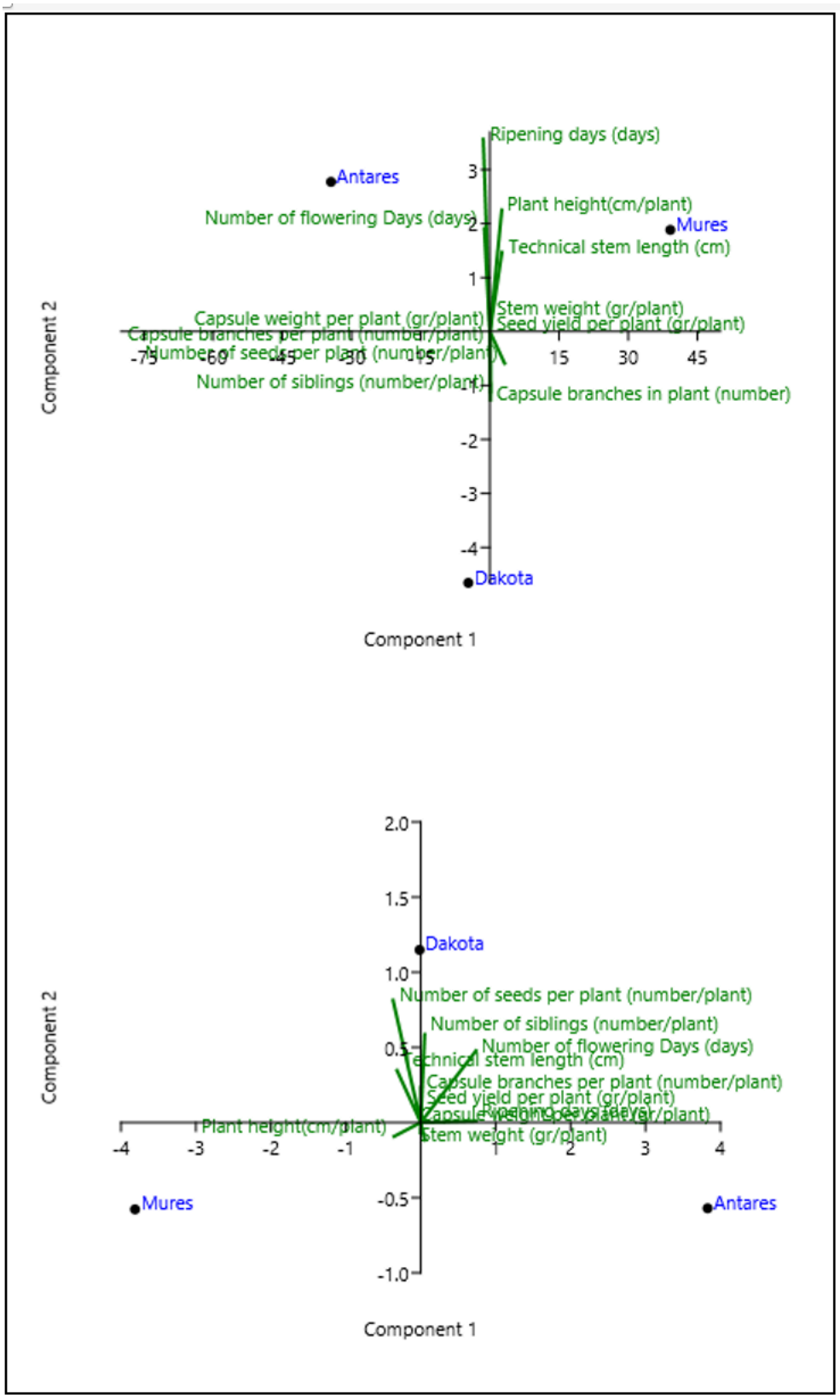

Figure 2. Principal Component Analysis (PCA) of growth and development parameters of flax cultivars used for treatments under the field (A) and the greenhouse conditions (B).

Heat-map clustering and Principal Component Analysis (PCA) of flaxseed oil and fatty acid compositions

For visualization, correlate and clarify the flaxseed oil and fatty acid compositions considered to the cultivars, we performed heat-map (Fig. 3) clustering and PCA (Fig. 4). In addition to the quantitative analysis of plant characteristics, the oil yield and fatty acid composition of seeds obtained from field and greenhouse conditions were also investigated. For the respective analysis, flax varieties characterised by high oil content (Antares, Dakota and Mures) were tested. Accordingly, oil yields ranged from 1.03, 0.94 to 0.56g for "Antares, Mures and Dakota" in the field and from 0.77, 0.68 to 0.41g in the greenhouse, respectively (Table 4). We analysed the ratio of quality and quantity indicators and the fatty acid composition of the oils after extraction. GC-FID analysis revealed that α-linolenic acid, linoleic acid, linoleic acid, palmitic acid, oleic acid and stearic acid were the major fatty acid components of the respective flax plant seed oils obtained both in the field and under greenhouse conditions. Considering the fatty acid percentages, the highest α-linolenic acid was found in Dakota (29.23%) and the lowest in Antares (20.53%) in the field, while the highest was found in Dakota (19.25%) and the lowest in Mures (16.13%) in the greenhouse. In linoleic acid, the highest was found in Dakota (19.57%) and Antares (11.37%) and the lowest in Antares (12.66%) and Dakota (9.44%) in the field and greenhouse, respectively. In addition, two components with Eigen values above 1 were observed under field conditions. These two components (F_1: 77.6% and F_2: 22.3%) explain a total variation of 99.9%. Accordingly, two components with Eigen values above 1 were observed under greenhouse conditions. These two components (F_1: 75.7% and F_2: 24.2%) explain a total variation of 99.9%.

Table 4. Oil and fatty acid compositions (%) of flaxseed varieties used in field and greenhouse treatments

Greenhouse fatty acids							
Variety	Oil content (g)	Crude oil yield (%)	Palmitic acid (C16.0)	Stearic acid (C18.0)	Oleic acid (C18.1)	Linoleic acid (C18.2)	α-Linolenic acid (C18.3)
Mures	0.68	13.6	6.11	7.42	13.11	10.28	16.13
Antares	0.77	14.4	7.14	8.2	12.54	11.37	18.73
Dakota	0.41	8.2	5.33	7.26	11.22	9.44	19.25
Field fatty acids							
Variety	Oil content (g)	Crude oil yield (%)	Palmitic acid (C16.0)	Stearic acid (C18.0)	Oleic acid (C18.1)	Linoleic acid (C18.2)	α-Linolenic acid (C18.3)
Mures	0.94	19.1	6.95	6.85	20.11	13.05	26.14
Antares	1.03	20.6	8.15	9.28	14.36	12.66	20.53
Dakota	0.56	11.7	6.13	10.32	10.04	19.57	29.23

Mechanical properties of flax fiber and scanning electron microscopy (SEM)

Due to the low fiber content of flax in relation to its fineness and strength, flax cannot compete with other fiber crops such as hemp and cotton. That caused gradual decreases in uses of flax for fiber. Regarding the fiber content, (Yılmaz and Uzun, 2019) reported the fiber content in flax stalk as 16-24% and 34-37%. In the present study, according to the results obtained from the analyses (tensile strength, puller strength, rupture stretched and fiber diameter measurement) on the varieties evaluated in the field and greenhouse; it was determined that Dakota varieties (1%) was the highest strain in greenhouse conditions and the lowest (0.58%) in field conditions and also the lowest (0%) in strain at break in the field. In addition, the highest tensile strength was found in Dakota (704.6 Mpa) and the closest Antares (692.2 Mpa) under field conditions, while the lowest was found in Dakota (198.5 Mpa) and Mures varieties (288.8 Mpa) under greenhouse conditions (Tablo 5). In addition, structural analyses were carried out with the help of Scanning Electron Microscopy (SEM) to determine the differences in the microstructures of the related varieties both in the field and greenhouse conditions. Additionaly, it was observed that the layers between the fiber and matrix were separated in the SEM images (Fig. 5).

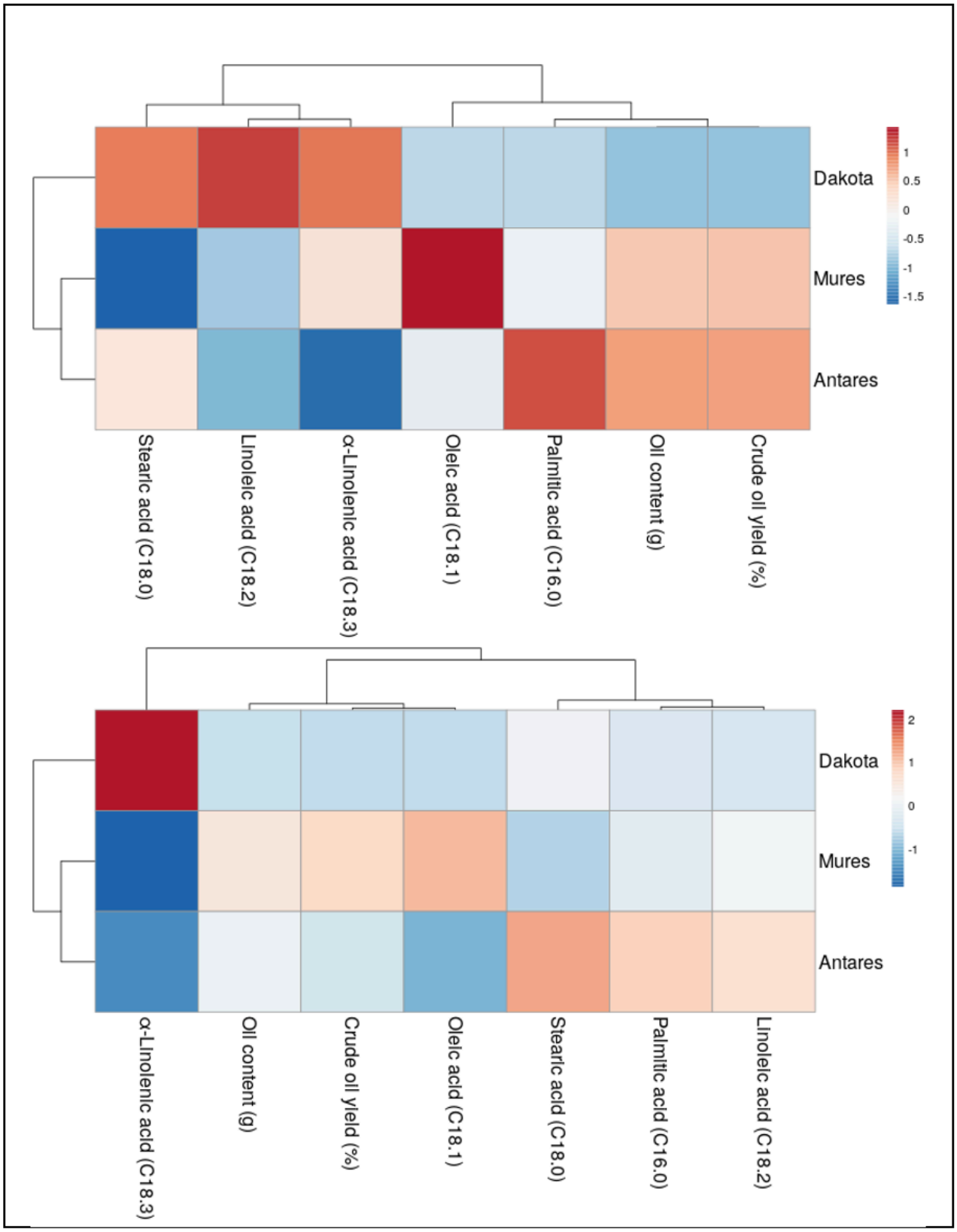

Figure 3. Heatmap clustering of oil and fatty acid compositions (%) of flaxseed cultivars used for treatments under the field (A) and the greenhouse conditions (B).

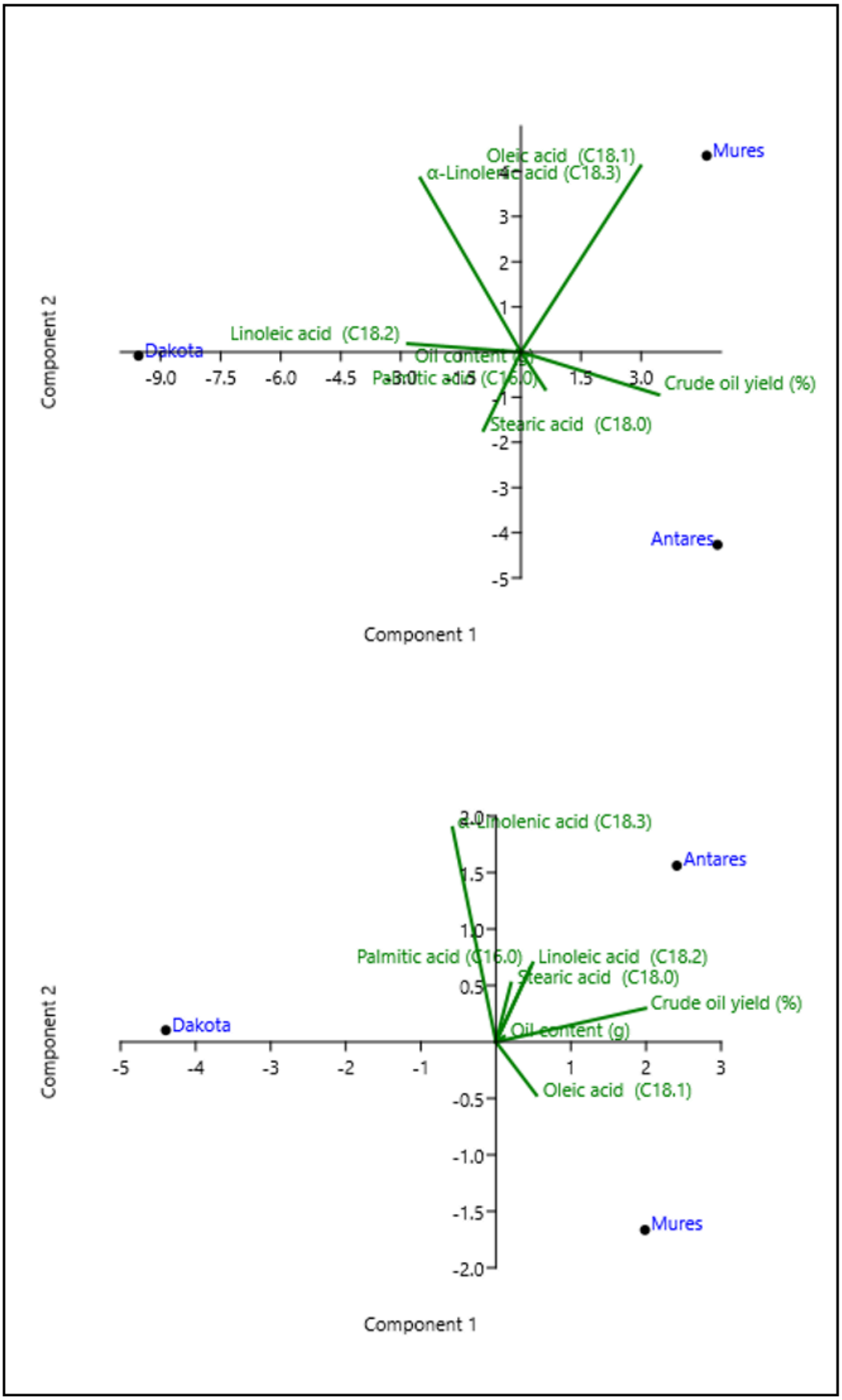

Figure 4. Principal Component Analysis (PCA) of oil and fatty acid compositions (%) of flaxseed cultivars used for treatments under the field (A) and the greenhouse conditions (B).

Table 5. Fiber mechanical properties of flax varieties used in field (A) and greenhouse (B).

Varieties	Growth conditions	Series (n=3)	Strain at fmax (%)	Tensile strength (Mpa)	Strain at break (%)
Dakota	field	mean value	0.58	704.6	1.2
	greenhouse	mean value	1	198.5	0
Mures	field	mean value	0.71	692.2	0.9
	greenhouse	mean value	0.73	288.8	1.2
Antares	field	mean value	0.83	587.4	1.2
	greenhouse	mean value	0.75	587.4	0.7

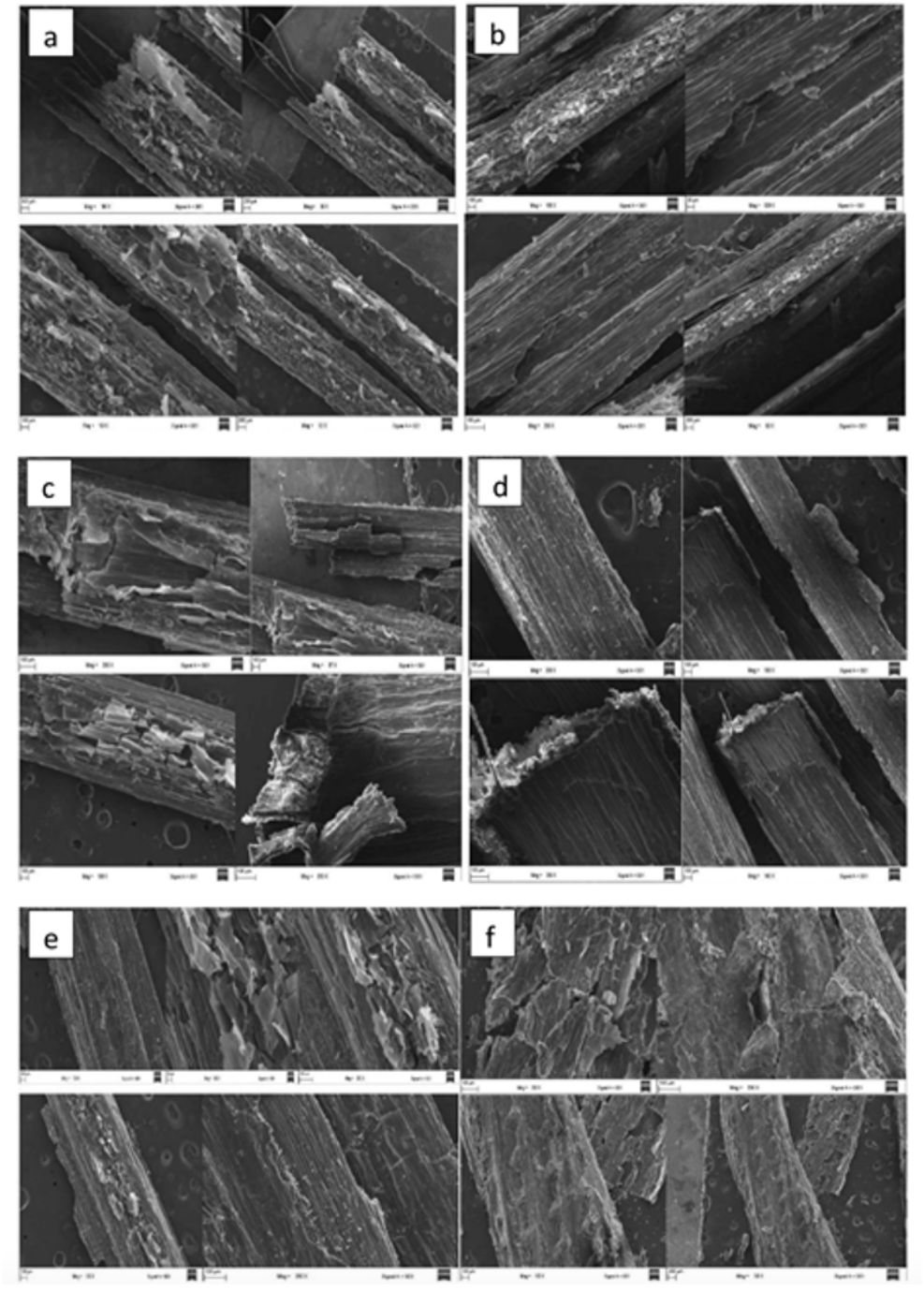

Figure 5. Scanning Electron Microscope (SEM) images the effect of growth conditions (field and greenhouse) on the flax varieties, **a).** Dakota (field); **b).** Dakota (greenhouse); **c).** Mures (field); **d).** Mures (greenhouse); **e).** Antares (field); **f).** Antares (greenhouse).

DISCUSSION

Choosing the right type of plant is critical in the face of everchanging environmental conditions. Due to the expanding world population and the increasing demand for food and materials, raw materials from the agricultural industry are increasingly in demand. In order to meet these increasing needs, we need to utilise productive and high quality varieties. In this context, flax, an important industrial plant with useful oil and fiber properties, has spread over a large part of the world. It is also one of the oldest cultivated plants (Melelli et al., 2022). In addition, it highlights the changes in plant height and technical stem height that affect the fiber structure and quality of the flax plant, as well as the fatty acid profile from the seeds obtained after harvest variety (Goudenhooft et al., 2018). These changes result in significant increases in plant height and thinness, the risk of lodging due to wind and rain, and significant changes in the fiber structure of the plants, which are the main characteristics that distinguish field crops from those grown in greenhouses (Li et al., 2022; Brulé et al., 2016). In addition, plants change in height and fineness, and even in fibrous plants such as flax, the structural performance and morphological characteristics of the fibers experience the same situation. At the same time, it is assumed that seismomorphogenesis is the only phenomenon that causes differences between the two states (Goudenhooft et al., 2019). In this context; flax, which is an important industrial plant with both oil, and fiber, properties, has its own distribution area in almost every geography of the world (Tian et al., 2021; Li et al., 2022; Melelli et al., 2022). In addition, the agro-morphological characteristics of the flax cultivars under field and greenhouse conditions, the fatty acid composition of the seeds and the fiber structure of the cultivars were studied by SEM. The flax varieties (Antares, Dakota and Mures) were tested for the first time for seismomorphogenesis in relation to their cultivation under field and greenhouse conditions.

High plant height, technical stem length and less branched structure are desirable for fibrous flax varieties. Plant height and technical stem length, which is a critical indicator/parameter, can be an important criterion in the selection of flax grown for fiber (Heller et al., 2015; Poudyal, 2017). According to the present results, the lowest value for plant height in flax was found in Antares (43.50 cm) and the highest value was found in Mures (79.50 cm), which is fibrous in field conditions. In addition, in greenhouse conditions this result is the lowest value for plant height was found in Antares (16.13 cm) and the highest value was found in Mures (18.36 cm). Our current findings in field and greenhouse conditions were found to be lower than previous reports (Goudenhooft et al., 2018). In addition, were found in parallel (Sheng et al., 2022). Furthermore, as regards the technical stem length of the flax varieties, the lowest value was found in Antares (39.60 cm) and the highest in Mures (76.10 cm) un-der field conditions; the lowest value was found in Antares (15.40 cm) and the highest in Dakota (17.50 cm) under greenhouse conditions (Goudenhooft et al., 2018). Our results were found to have lower values compared to previous studies. In this context, the plant height and technical stem lengths of the varieties used in the field and greenhouse were lower than in previous reports, in line with our present findings, it was found that there were significant differences in the fiber structures of the varieties in the field and in the greenhouse. The results of flax fiber tensile strength (Mpa) and strain at break (%) were found to be lower compared to previous studies (Chuah et al., 2014). In particular, quality parameters such as fiber strain at fmax (%) tensile strength (Mpa) and strain at break (%) were reported to be important. It was also emphasised that quality parameters may be related to the matrix between the fibers (da Silva et al., 2023). According to the measurements made on the technical system and the SEM images, it was found that the fiber breaking stress values (0-1,2%) of the related plant under field and greenhouse conditions were lower than previous studies. Furthermore, it is stated that the mechanical properties of the matrix structure in the fiber structure of flax deteriorate due to hydrolysis and the layers between the fiber and the matrix are separated, especially as a result of this separation, rupture and fracture increase, as well as the strain at break is significantly negatively affected (Moudood et al., 2019).

As expressed in this study; the oil yields of the cultivars varied between 11.7-20.6% under field conditions and 8.2-14.4% in the greenhouse, respectively; the field conditions were similar to previous studies, while those in the greenhouse were found to be low (Keskin et al., 2020; Njembe et al., 2021). In addition, α-linolenic acid (20.53-29.23%; 16.13-19.25%) and linoleic acid (12.66-19.57%; 9.44-11.37%) were observed in the field and greenhouse conditions, respectively. In this context, α-linolenic acid was determined as 48.41% (Hatanaka et al., 2021) and linoleic acid as 14.90% (Xie et al., 2020; Njembe et al., 2021). The present findings were found to be lower than previous studies. As expressed, it has been reported that agro-morphological traits are interdependent and show differences especially when evaluated as oil and fiber (Mirshekari et al., 2012). In this context, it was found that the differences determined in agro-morphological traits observed in the related plant in our results taken under field and greenhouse conditions may be related to soil organic matter content, pH and lime (Pisupati et al., 2021), and it was found that our results are different from previous studies and compatible with the literature; in addition, it is predicted that these differences may be the quality of the seed used and the climatic conditions of the growing environment. In contrast, field-grown plants are actually more exposed to a wide range of other factors, such as rainfall or greater temperature fluctuations, which can influence many climatic factors. For this reason, growing flax in greenhouses may be of interest to avoid yield losses due to lodging, severe weather events or other undesirable factors, while maintaining fiber properties.

In addition, growing conditions can be controlled in the greenhouse, which is not the case in the field. This control is important because it can allow several har-vests per year, thus increasing the availability of flax fiber to the industry while providing fiber with similar positive properties.

CONCLUSIONS

In response to stresses of mechanical origin, such as wind, the shape and mechanical parameters of plants can be greatly affected. Moreover, in the case of the flax, plants those grown in greenhouses have specific shape parameters that differ significantly from those of plants grown in the field. In fact, when flax is subjected to a much milder mechanical stress, both the structure of the whole plant (such as the fiber) and the height of the technical stems increase considerably. Due to these shapes, the flax fibers are distributed differently along the stems. In the findings of this study, the highest plant height and technical stem length of the registered cultivars (Antares, Dakota, Mures) in terms of fiber-related traits were observed in the Mures cultivar under field and greenhouse conditions, respectively. In addition, it is predicted that varieties with fiber structure characteristics will be grown in ecologically suitable regions, that field conditions will have high morphological values compared to green-houses and that fiber structures will be stronger.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

All data created and analyzed during the experiments are presented in this study.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Effects of shilajit addition to honey bee diet on semen freezing

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Abstract
This study aimed to investigate the effects of adding shilajit to the diet of honey bees on semen freezing. A total of 5 groups were formed in the research, one of which was a control group (SH-0) and the other four were an experimental group (SH-1, SH-2, SH-3, SH-4). A total of 25 study colonies were used, 5 in each group. While the SH-0 group was formed without using any additives, the experimental groups were fed with the addition of shilajit (1/1 sugar/water) in different doses (5, 10, 15, 20 mg/L) to the honey bee diet. The collected semen samples were frozen in liquid nitrogen vapor and then stored in liquid nitrogen at -196°C. Then, semen samples were thawed at 37°C and evaluated to determine spermatological parameters (motility, acrosome integrity, plasma membrane integrity, spermatozoa concentration). It was also examined in terms of total oxidant and total antioxidant. Compared to the control group, it was determined that all shilajit doses significantly increased spermatological parameters such as motility, hypoosmotic swelling test (HOST), acrosome integrity ($p<0.001$), but did not significantly affect the spermatozoa concentration value ($p>0.05$). Although there is no statistical difference between the groups in terms of semen TAS (Total Antioxidant Status) and TOS (Total Oxidant Status) values, which are oxidative stress parameters, the numerical increase in TAS values in SH-2 and SH-3 groups; striking. As a result, it was determined that adding shijajit to the honey bee diet positively affected the post-thawing spermatological parameters of frozen bee semen.
Keywords: Antioxidant, Freezing, Honey bee, Shilajit, Semen

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INTRODUCTION

To increase the yield characteristics of honey bees (*Apis mellifera*), appropriate colonies should be obtained to benefit from artificial insemination, to provide genetic improvement and to carry out breeding studies. It is known that gene errors that cause offspring death may occur as a result of the increase in the consanguinity status of the colonies (Danka et al., 1986). To prevent inbreeding and to prevent diseases caused by the queen bee, it is necessary to add blood from colonies with appropriate breeds and desired characteristics. For this purpose, it is very important that honey bee semen can be stored. It has been reported that great success has been achieved in artificial insemination applications recently (Cobey, 2007).

Cryopreservation methods are used for the long-term storage of honey bee semen. For this purpose, semen can be stored in liquid nitrogen for the short or long term by using various cryoprotectants such as dimethyl sulfoxide (DMSO) and glycerol (Harbo, 1979). However, it has been determined that queen bees fertilized with semen stored for a long time lay a large number of unfertilized

eggs. This situation is thought to be due to genetic damage (oxidative stress) during the freezing of semen (Harbo, 1981). Oxidative stress is defined as the harmful effects of ROS (reactive oxygen species) due to antioxidant deficiency, which is the body's natural defense mechanism (Tremellen, 2008). Petruska et al. (2014) emphasized that antioxidants added to semen used in artificial insemination in farm animals are important for reducing the negative effects caused by ROS.

Shilajit has been widely used in medicine for many years. Shilajit has antioxidant and anti-inflammatory properties with the active ingredients it contains. It has been stated that fulvic acid in the structure of shilajit causes a decrease in the abnormal semen rate and malondialdehyde (MDA) level, in addition, it protects the integrity of the membrane and acrosome and causes an increase in motility (Xiao et al., 2018). Stohs (2014) stated that shilajit has antioxidant, anti-inflammatory, immunomodulatory, adaptogenic effects as well as positive effects on spermatogenesis. Moreover, Biswas et al. (2010) reported that shilajit had a positive effect on oxidative stress in oligospermic patients, causing a decrease in MDA levels and a positive effect on motility,spermatozoa concentration and semen volume. Furthermore, Mishra et al. (2018) stated that shilajit is effective against the negativities caused by toxic substances.

In this study, it was aimed to determine the effects of shilajit added to the honeybee diet on the long-term storage of honeybee semen.

MATERIAL AND METHODS

In this study, a total of 5 groups were generated, one of which was the control (SH-0) and the other four (SH-1, SH-2, SH-3, SH-4) were the experimental groups. A total of 25 study colonies, 5 in each group, were used. In the present study, 10 support colonies were used to provide drone larvae of the colonies and to meet the worker bee needs. The control group was fed with no additives, and the experimental groups were fed with the honey bee diet (1/1 ratio sugar/water) with the addition of shilajit in different doses (5, 10, 15, 20 mg/l). Drone slats from support colonies were transferred to working colonies, where the mature drones were collected to collect semen when they were 2-3 weeks old. By applying pressure to the thorax and abdomen of the drones, the endophallus was exposed and semen was collected. At the beginning of the study, a motility evaluation was performed in native semen, and it was observed that the mean of motile spermatozoa rate was above 80%. To dilute semen, Modified Kiev Solution (0.3 g D^+-glucose, 0.41 g KCl, 2.43g sodium citrate dihydrate per 100 ml) (Rosseau and Giovenazzo, 2016), was prepared containing 10% DMSO. Reconstituted semen was drawn into 0.25 mL straws in accordance with the diluent-air-space-semen-air-space-diluent procedure, and the tip of the straws was pressed and closed. Straws were kept at 4ºC for 2 hours for equilibration. Straws were frozen in liquid nitrogen vapor for 10 minutes at approximately 5 cm above the liquid nitrogen assembly. The frozen straws were transferred to the liquid nitrogen tank for long-term storage. Then, thawing was performed at 37 ºC for 30 seconds to evaluate the semen samples. Thawed semen were examined in terms of motility, plasma membrane integrity (HOST), spermatozoa concentration, acrosome integrity, total antioxidant level (TAS) and total oxidant level (TOS) parameters. Motility was determined as 20%, 40%, 60%, 80%, taking into account the intensity of the circular movement specific to honeybee semen (Taylor et al., 2009., Gontarz, et al 2016). To determine the HOST positive rate, 10 µl of semen were kept in 100 µl of 100 mOsm HOST solution at 37°C for 30 minutes. 200 spermatozoa were counted and spermatozoa with coiled, bent tails were evaluated as HOST positive (Alçay et al., 2019). In order to evaluate the concentration, of spermatozoa, 1 µl of semen was diluted with 1 ml of Kiev diluent, then placed on a Thoma counting chamber and counted 5 middle squares at 400x magnification under the microscope and calculated according to the formula below (Tekin, 1994; Cobey, 2013). To determine the acrosome integrity, 10 µl of semen was added to 100 ml of phosphate buffer saline (PBS), it was centrifuged at 100 RCF for 5 minutes and the supernatant was separated and discarded. PBS in 100 ml was added to the remaining semen and centrifuged at 100 RCF for 5 minutes. Again, the supernatant was discarded. A smear was taken from the semen below and stained with PSA-FITC solution in the darkroom. After keeping it in the dark at 37°C for 1 hour, at least 200 spermatozoa were examined under a fluorescent microscope and the acrosome integrity was determined as % (Alçay et al., 2019). Spectrophotometric (Thermo®) measurements were made using special kits (REL Assay Diagnostics®) to determine TAS and TOS values, which are oxidative stress parameters.

Statistical analysis of data obtained in the present study (One-way ANOVA) and comparisons between groups (Duncan's test) were performed using SPSS package program (SPSS Inc., Chicago, IL, USA). The effects (significance) of the groups were evaluated at $P<0.05$ level (IBM., Corp., 2011). The number of colony used in the study was determined by G*Power (Faul et al., 2007) software package (version 3.1.4).

RESULTS AND DISCUSSION

Motility, spermatozoa concentration, HOST, acrosome integrity (Table 1), total antioxidant and total oxidant (Figure 1) values were determined by adding different doses of shilajit (0, 5, 10, 15, 20 mg/l) to bee diet in honey bee colonies.

The effects of shilajit in different amounts added to the honey bee diet on the cryopreservation of bee semen were

examined. In our study, the average motility values of the groups (SH-0, SH-1, SH-2, SH-3, SH-4) were 20%, 56%, 52%, 60% ,and 64%, respectively (Table 1). There was a significant difference between the groups in terms of motility values (%). It was determined that adding different doses of shilajit to the honey bee diet significantly increased the motility percentage ($P<0.001$).

Findings regarding the effects of shilajit at 0, 5, 10, 15, 20 mg/l levels on the average spermatozoa concentration, in honey bee semen are given in Table 1.

HOST (the Hypo-Osmotic Swelling Test), known as the plasma membrane integrity test, is an important test for evaluating the functional integrity of the membrane structure of spermatozoa. The increase in the positive rate of the test is an indication that the spermatozoa have a solid membrane structure. In our study, the average positive percentages of the groups (SH-0, SH-1, SH-2, SH-3 and SH-4) of frozen honey bee semen were 46.20%, 67.20%, 72.20%, %74.20, % 79.20 was determined as 79.00% (Table 1). It was also observed that the addition of shilajit to the honey bee diet significantly increased the HOST positive percentage ($P<0.001$). In a study, it was emphasized that Reactive Oxygen Species (ROS) cause lipid peroxidation in the lipid sperm membrane and that this may lead to deterioration of the sperm membrane and decrease in motility, and that the negative effects of ROS on the sperm cell can be prevented by the use of antioxidants (Liu et al., 2015). When the statistical average values were examined, it was found important that as the amount of shilajit used increased, the percentage of membrane permeability also increased. In the study, it was also found remarkable that there was a general increase in the rate of motile spermatozoa in parallel with the increase in host values. This shows that shilajit improves plasma membrane integrity as well as sperm motility.

There was a significant difference between the groups in terms of positive values of acrosome integrity ($P<0.001$). SH-0 (96.60%), SH-1 (97.80%), SH-2 (98.20%), SH-3 (98.80%) and SH-4 (99.20%) average as can be seen from the values, acrosome integrity values gradually increase as the amount of shilajit added to the syrup increases (Table 1). Therefore, it was observed that the addition of shilajit significantly increased the acrosome integrity percentage ($p<0.001$).

Table1. Post-thaw spermatological parameters in study groups

Groups	Motility (%)	Spermatozoa Concentration (x10⁴/µl)	HOST (%)	Acrosome Integrity (%)
SH-0 (0mg/L)	20.00±0.00^{b}	425.00±48.70	46.20±2.15^{c}	86.60±0.24^{d}
SH-1 (5mg/L)	56.00±4.00^{a}	400.00±32.55	67.20±2.05^{b}	87.80±0.20^{c}
SH-2 (10mg/L)	52.00±4.89^{a}	515.00±76.85	72.20±0.66^{ab}	88.20±0.20^{bc}
SH-3 (15mg/L)	60.00±0.00^{a}	590.00±38.40	74.20±1.71^{ab}	88.80±0.20^{ab}
SH-4 (20mg/L)	64.00±7.48^{a}	485.00±16.95	79.00±5.83^{a}	89.20±0.20^{a}
P	<0.001	0.051	<0.001	<0.001

a,b,c,d: Differences between means with different letters in the same column are significant. SH-0: Control; **SH-1**: 5 mg/L shilajit; **SH-2**: 10 mg/L shilajit**; SH-3**: 15 mg/L shilajit**; SH-4:** 20 mg/L shilajit. **HOST**: Hypoosmotic Swelling Test; ±: Standard Deviation

The effects of shilajit, which was added to the honey bee diet at different levels, on semen TAS values are given in Figure 1. When the data were examined, the mean TAS values of the groups were determined as 11.50, 4.50, 14.50, 14.50, and 8.00, respectively. It was noteworthy that the semen TAS values of the SH-2 and SH-3 groups were numerically higher than the other study groups. TOS values of the groups were found as 98.45, 87.69, 123.07, 110.76, 55.38, respectively (Figure 1).

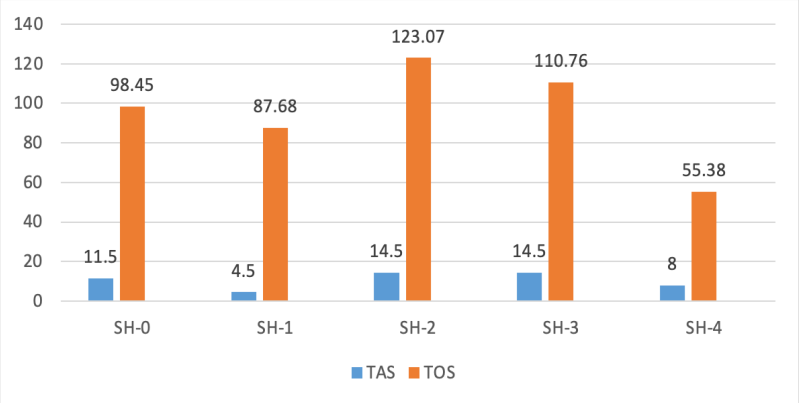

Figure 1. Post-thaw TAS and TOS parameters in semen in study groups

SH-0: Control; SH-1: 5 mg/L shilajit; SH-2: 10 mg/L shilajit; SH-3: 15 mg/L shilajit; SH-4: 20 mg/L shilajit.

In this study, spermatological parameters (motility, sperm concentration, HOST, acrosome integrity) and oxidative stress parameters (TAS, TOS) were investigated to determine the effects of shilajit added to the honey bee diet at doses of 0, 5, 10, 15, 20 mg/L on long-term storage of honeybee semen.

Taylor et al. (2009), in their study on the comparison of different diluents in the freezing storage of semen, defined the motility values as 0, 20, 40, 60, 80 and above 95, but expressed them with values between 0 and 5. Diluent 3 and diluent 4 used in the study are hypotonic Diluent 3 and diluent 4 used in the study are hypotonic. Diluent 3 is a Tris extender containing amino acids. Diluent 4 is a modified Kiev extender containing antioxidants. It was observed that the motility values of diluent 4, which was more successful than other diluents, were above diluent 3. It has been emphasized that this situation may be due to the antioxidants used. Shilajit used in different doses in our study showed improvement in spermatological parameters with increasing doses due to its antioxidative effect. It has been emphasized that this situation may be due to the antioxidants used. The use of Kiev solution and a diluent containing 10% DMSO in the study is similar to our study. Additionally, the study showed that DMSO was significantly more effective than other cryoprotectant agents. Therefore, the Kiev solution and 10% DMSO used in the study coincide with our current study. The results of experimental group 4, whose motility percentage was successful compared to the other groups in the study, are similar to the average findings of the SH-3 and SH-4 groups (60%, 64%) in our current study.

Biswas et al. (2010) in their study examining the effect of shilajit on motility by spermiogram test, they emphasized that the motility values of patients with oligospermia who received oral shilajit treatment for 3 months increased significantly. In the study, the importance of the effect of shilajit on motility was emphasized by comparing the motility values before the treatment with the sperm results collected at 0.5, 1 and 2 hours after the treatment. While an average increase of 12.4% in motility was observed in the first half hour, an increase of 13.2% in 1 hour and 1.4% in 2 hours was observed, and it was stated that shilajit had a significant effect on motility.

It has been reported that selenium and various mineral substances in the structure of shilajit, dibenzo-alpha pyrons, as well as humins containing humus, humic acid and fulvic acid have regulatory and antioxidant effects (Carrasco-Gallardo et al. 2012). It has been stated that shilajit has an aphrodisiac effect (Ghosal, 1990). Moreover, it has been emphasized that fulvic acid caused a decrease in abnormal spermatozoa and malondialdehyde levels at a certain rate, so the use of shilajit caused an increase in motility (Xiao et al., 2018). It has been reported that shilajit increases semen motility by increasing the testosterone level and the number of spermatozoa in the epididymis (Park et al., 2006). Kreuter et al. (2002) stated that fulvic acid plays an important role in providing energy to spermatozoa, therefore it is an element that increases motility. Studies on shilajit-related motility show that shilajit has a positive effect on motility values, which seems consistent with our study.

Studies in mammals have shown that shilajit increases semen concentration. Park et al. (2006) emphasized the importance of shilajit increased the number of epididymal spermatozoa in rats compared to the control group, thus contributing significantly to the spermatogenesis process.

As a result of the increase in polyunsaturated fatty acids in the spermatozoa plasma membrane, the increase of free radicals and the creation of a suitable environment for reactive oxygen species cause the structure of the spermatozoa to deteriorate, resulting in a decrease in the number of spermatozoa (Alvarez and Storey, 1995). It has been emphasized that shilajit significantly decreases the MDA level in semen and improves semen quality (Biswas et al., 2010). It is also known that shilajit increases the testosterone level required for spermatozoa production (Park et al., 2006).

It is thought that the reason why no statistically significant difference was found between the concentration values of honey bee sperm in the studies is due to the unique structure of honey bee sperm and the evaluation of bee sperm by creating a sperm pool rather than individually for each bee.

Wegener et al. (2012) emphasized that the mean value of 65.3±12 in unfrozen semen and 30±2.1 in frozen-stored semen was insignificant as the percentage of impermeable cells when evaluating osmotic stress in their study on honey bee semen parameters. In the present study, the gradual increase of the HOST positive rate starting from the control group is important and does not coincide with the study values. It is thought that the reason for this situation may be due to the efficacy of shilajit as well as the difference in semen dilution rate and the HOST determination method. In another study on oxidative stress in honey bees, Alçay et al. (2019) investigated the effects of different amounts of the diluent containing TL-hepes supplemented with BSA on osmotic stress in honeybee semen. Although the HOST values were close to each other in the study, the percentage values of the HOST positive rate in numerical terms increased starting from the control group (59.20 ± 3.58%), and it was determined as BSA5 (67.73±4.07) was the highest group. Our current study observed that with increasing levels of shilajit added to the bee diet (5 mg, 10 mg, 15 mg, 20 mg), the percentage of HOST positivity significantly increased (67.20, 72.20, 74.20, 79.00). The study observed that the groups participating in shilajit increased significantly compared to the control group (46.20%).

In addition, the current study found that adding shilajit to the honey bee diet is important. When the current study is compared with the studies, it is seen that the statistical mean values are similar. Although there is no study on shilajit in honey bees, it is seen that the results of the studies on mammals are similar to our study. Kumar et al. (2018) emphasized that the HOST positivity rate in frozen-thawed semen in buffalos treated with shilajit increased significantly after treatment (57.6±0.9%) compared to before treatment (39.8±0.57). Kumar's positive effect of shilajit on buffalo semen is similar to the effect of shilajit on bee semen in the presented study. It has been mentioned that fulvic acid has a curative effect on cell functions, especially preventing damage to mitochondria and nuclei. It does this by suppressing the effect of free radicals (Sultan et al., 2021). It has been emphasized that with components such as quinone-semiquinone-hydroquinone complex, catalase, superoxide dismutase, and glutathione peroxidase in the structure of shilajit, lipid peroxidation can be prevented that may occur during freezing and thawing of semen (Agarwal et al., 2007). It has been reported that shilajit prevents the formation of lipid peroxidation, which is known to disrupt the membrane structure of spermatozoa (Tripathi et al., 1996). In our study, it is predicted that the positive status in HOST values is due to the components that prevent the formation of lipid peroxidation and high antioxidant content in the structure of shilajit.

Although there are many publications on honey bee semen, Alçay et al. (2019) investigated the protective effects of TL-hepes based diluent supplemented with BSA in their study on acrosome defect in frozen semen. In the study, it was seen that the most successful group was 54.33±3.71 %. In our study, it was observed that shilajit was significantly effective in preserving acrosome integrity. Kumar et al. (2018), in their study evaluating the effects of shilajit on buffalo semen, applied shilajit treatment orally to buffaloes for 2 months and acrosome integrity in frozen and thawed semen before treatment (40.9±0.72%), in thawed semen (49.6±1.51%) and thawed semen after treatment (48.9±0.60) was found significant. Studies on acrosome integrity are similar to our study on the effectiveness of shilajit.

No studies were found on TAS and TOS in honey bee semen. It is known that enzymatic antioxidants have positive effects on semen. Studies have emphasized that catalase has positive effects on semen (Taylor et al., 2009; Weirich et al., 2002). Shilajit contains selenium and various mineral substances, dibenzo-alpha pyrons as well as humins containing humus, humic acid and fulvic acid. The fulvic acid in the structure of shilajit contains antioxidant and anti-inflammatory substances (Carrasco-Gallardo et al., 2012). It has been observed that fulvic acid protects the spermatozoon membrane structure and acrosome integrity, and causes an increase in motility. It has also been emphasized that it causes a decrease in abnormal spermatozoa rate and malondialdehyde level (Xiao et al., 2018).

CONCLUSION

Studies show that the amount of MDA (malondialdehyde), which is important for oxidative stress, is significantly reduced with the use of shilajit, and that shilajit has a positive effect against oxidative stress. As a result of the study, it was observed that adding shilajit to the honey bee diet ensures long-term storage of honey bee semen and has a positive effect on spermatological parameters (motility, plasma membrane integrity, acrosome integrity). Research results reveal that feeding bees with shilajit will provide longer storage conditions for honeybee semen. Such studies on honeybee semen may contribute to improving longer-term storage conditions. As a result, it has been understood that shilajit has distinct advantages in long-term storage of honey bee sperm, especially in preserving motility, HOST and acrosome integrity.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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