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Researches on feeding behaviors of *Nezara viridula* (Linnaeus, 1758)(Hemiptera: Pentatomidae) adults in intermittent CO_2 applications

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DergiPark AKADEMİK

Abstract

In this study, the effects of 3 different doses (430, 600 and 670 ppm) of intermittent CO_2 applications on the feeding behaviors of *Nezara viridula* L. (Hemiptera: Pentatomidae) Adults were determined. As nutritional behavior, the total nutrition duration of adult male and female individuals other than nutrients was examined. As a result of the study, no statistically significant difference was found between the total feeding times of female and male individuals of *N. viridula***.** A statistically significant difference was found between the duration of stay on the nutrients of female and male individuals of *N. viridula* who received 600 ppm and 670 ppm doses of CO_2. The duration of stay on the nutrients of male individuals who received 600 ppm and 670 ppm doses of CO_2 was found to be significantly lower than the duration of stay on the nutrients of female individuals. The duration of stay on the nutrients of male individuals who received 670 ppm doses of CO_2 was found to be significantly lower than the duration of stay on the nutrients of female individuals. No statistically significant difference was found between the CO_2 dose groups of *N. viridula* and the total feeding times of the control group. A statistically significant difference was found between the CO_2 dose groups of *N. viridula* and the duration of stay apart from the nutrients and the duration of stay on the nutrients of the control group. The duration of stay on the nutrients of individuals exposed to 600 and 670 ppm CO_2 doses was found to be significantly lower than the duration of stay on the nutrients of individuals in the control group. In addition, the duration of stay apart from the nutrients of the individuals to whom 600 and 670 ppm CO_2 doses were applied was found to be significantly higher than the duration of stay apart from the nutrients of the individuals in the control group. As a general result of this study, it was determined that as the application dose increased, the feeding behaviors of male and female individuals changed, male individuals reacted more to dose administration and remained on nutrients less than female individuals.

Keywords: *Nezara viridula*, Feeding Behaviors, Intermittent CO_2

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INTRODUCTION

Southern green stink bug, *Nezara viridula* (Linnaeus, 1758) (Hemiptera) (Southern green stink bug), one of the important pests belonging to the Pentatomidae family, is a polyphagous and cosmopolitan pest (Figure 1). *N. viridula*, which has spread to many parts of the world, is spreading to new areas because it is well adapted to changing climatic conditions and has the ability to fly well (Lye and Story, 1988; Panizzi, 1997; Çetin and Karsavuran 2000; Birgücü, 2012).This pest, which is widely found in our country (Lodos, 1986; Lodos et al.,

1998), can be suppressed by its natural enemies under normal conditions. In addition to legumes, the pest is also fed on tomatoes, peppers, beans, hemp, cotton, soy, sesame, alfalfa, tobacco, nuts, citrus fruits, other fruit trees and many wild and cultivated plants (Lodos, 1986; Lodos et al., 1998). With unconscious agricultural pest control practices, it causes serious economic losses in areas where there are no natural enemies. In an environment where there is no natural enemy pressure, the pest population causes significant economic damage (Todd, 1989). With global warming, it is thought that the populations of pests will increase in the next 50 years. Because; atmospheric gases have significant effects on harmful-hostile and natural enemy species. CO_2 has an important place in these gases. How and to what extent the change effects of the amounts of carbon dioxide with global warming will be determined should be determined by sample simulation studies. In the coming years, changes in atmospheric gas levels, especially with temperature change, will cause pests to form different biotopes. These biotopes are expected to increase their damage status by being affected by physiological changes in the plant (Vailla et al., 2019). This study was conducted to determine the effects of CO_2 applied in three different doses to *N. viridula* adults reared under laboratory conditions on the adult feeding behaviors.

Figure 1. *Nezara viridula* adults being reared

MATERIALS AND METHODS

Mass Rearing of *Nezara viridula* and Determination of Feeding Behaviors

Insect rearing and trials were carried out in a climate room prepared according to conditions with a temperature of 25±1ºC, a humidity of 45±5%, and a lighting time of 16:8 hours (Figure 2). The cultures were fed with tomato, tobacco and Datura seeds, sunflower seeds, green beans, peanuts and soya beans.

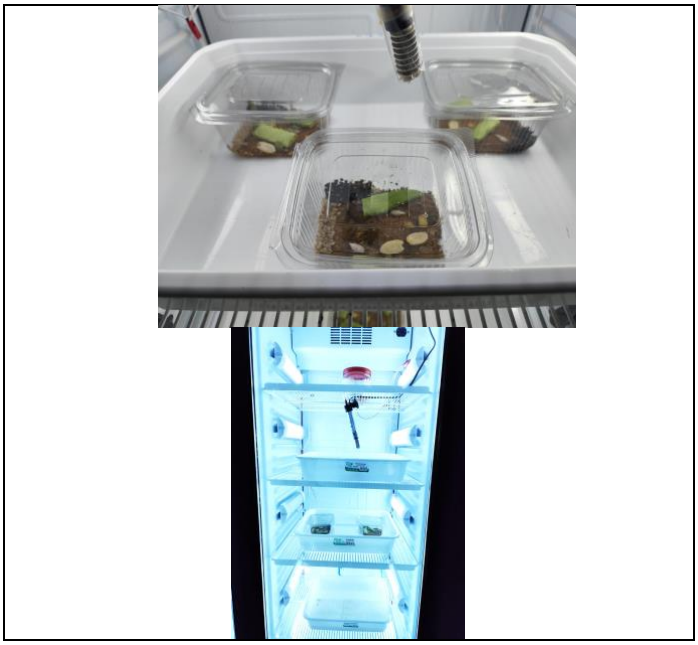

Figure 2. Mass rearing conditions of *Nezara viridula*

In the study, a total of 30 adult individuals, 5 adult males and 5 preoviposition females and 5 adult (female/male) controls, were used in *Nezara viridula* adults for each dose. The experiments were repeated with 10 replicates for each of the three CO_2 doses including the control treatment and 30 individuals for each of the three periodic feeding observations, totalling 90 replicates. Individuals were observed by administering carbon

dioxide in two different doses (600 and 670 ppm) in the CO_2 unit (Figure 3) where controlled conditions were provided. The applications were compared with the control (430 ppm), and the nutritional behaviors of the adults were observed and recorded in the cage within 1 hour in the observations made through the camera. Observations were recorded in seconds (3600 seconds). During the observation period, the duration of stay apart from the nutrients, the duration of stay on the nutrients and the total feeding time of the individuals were determined separately by the camera and eye control placed in the cabin in seconds (sec.).

Figure 3. Carbondioxide unit of applications

The Periods Determined for Nutritional Behavior;

Duration of Stay Apart From The Foods: The time taken for activities of *Nezara viridula* individuals such as waiting, resting, and foraging,

Duration of Stay on The Foods: The time elapsed until it extends its hose to the nutrients and/or leaves the nutrients without being fed on the nutrients,

Total feeding time: The time it takes from the moment it starts to extend vertically to insert its hose into the nutrients until it pulls its hose out of the nutrients

Statistical Analysis

"Shapiro-Wilk Goodness-of-Fit Test and Kolmogorow-Smirnov test" were used to test whether the obtained data fit the normal distribution. Descriptive statistics such as mean, median and standard deviation are given in the data in the study. Since it does not meet the normality assumptions, Mann Whitney U test was used to compare binary groups, and Kruskal Wallis Analysis was used to compare more than two groups. In all analyses, the statistical significance level was accepted as $p<0.05$. Statistical analyzes were performed using SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) 22 package program.

RESULTS AND DISCUSSION

Statistical analyzes of the distribution of the duration of *Nezara viridula* adult individuals on and apart from the nutrients at two different carbon dioxide concentrations in a 1-hour period according to male and female individuals are shown in Table 1.

Table 1. Statistical comparisons of different feeding behaviors of *Nezara viridula* adult individuals

	Dosage (ppm)	Gender	N	Mean (sec.)	Std. Deviation	Median	U	*P* value
Total Feeding Time	600	Male	5	1430,00	74,161	1410,26	20,00	0,117
		Female	5	1552,43	126,222	1580,50		
	670	Male	5	1316,28	90,229	1350,30	19,00	0,173
		Female	5	1390,36	102,901	1420,30		
Duration of Stay On The Foods	600	Male	5	150,63	9,587	151,30	25,00	**0,009**
		Female	5	177,55	8,742	179,44		
	670	Male	5	142,90	9,378	142,20	25,00	**0,009**
		Female	5	182,39	6,983	181,40		
Duration of Stay Apart From the Foods	600	Male	5	823,67	6,934	820,66	16,00	0,548
		Female	5	828,26	12,548	832,36		
	670	Male	5	824,06	13,219	822,50	22,00	**0,047**
		Female	5	843,25	9,915	842,50		

When Table 1 was examined, no statistically significant difference was found between the female and male groups of *N. viridula* in terms of total feeding times (p>0.05). A statistically significant difference was found between the female and male individuals of *N. viridula* in terms of the duration of stay on the nutrients and the duration of 670 ppm apart from nutrients (p<0.05). The duration of stay on the nutrients of male individuals who received 600 ppm and 670 ppm carbon dioxide doses was significantly lower than the duration of stay on the nutrients of female individuals (p<0.05). The duration of stay apart from the nutrients of male individuals who received 670 ppm carbon dioxide doses was significantly lower than the duration of stay apart from the nutrients of female individuals (p<0.05).

The statistical results showing the differences in the duration of *Nezara viridula* adult individuals on and apart from nutrients at two different carbon dioxide concentrations in a 1-hour period compared to the control are shown in Table 2.

Table 2. Statistical Comparison of Different Nutritional Behaviors of *Nezara viridula* Adult Individuals with Control Applications

	Groups	n	Mean (sec.)	Std. Deviation	Median	H	*P*value
Total Feeding Time	Control	10	1772,50	58,27	1780,50	21,399	0,117
	D600	10	1491,22	116,99	1495,40		
	D670	10	1353,32	99,24	1365,40		
Duration of Stay On The Nutrients	Control	10	203,06	30,53	196,25	8,715	**0,013**
	D600	10	164,09	16,62	164,23		
	D670	10	162,64	22,22	164,35		
Duration of Stay Apart From The Nutrients	Control	10	738,56	14,62	734,45	20,042	**0,000**
	D600	10	825,96	9,86	825,50		
	D670	10	833,65	14,95	833,55		

When Table 2 was examined, the differences between the total feeding times of *N. viridula* were not statistically significant according to the application groups (p>0.05). The differences between the duration of stay on the nutrients of pests were found to be statistically significant according to the application groups (p<0.05). It was observed that the duration of stay apart from the nutrients of pests was statistically different according to the application groups (p<0.05).

Boxplot graphs demonstrating the differences between control and dose applications of the pest are shown in Figure 4-6.

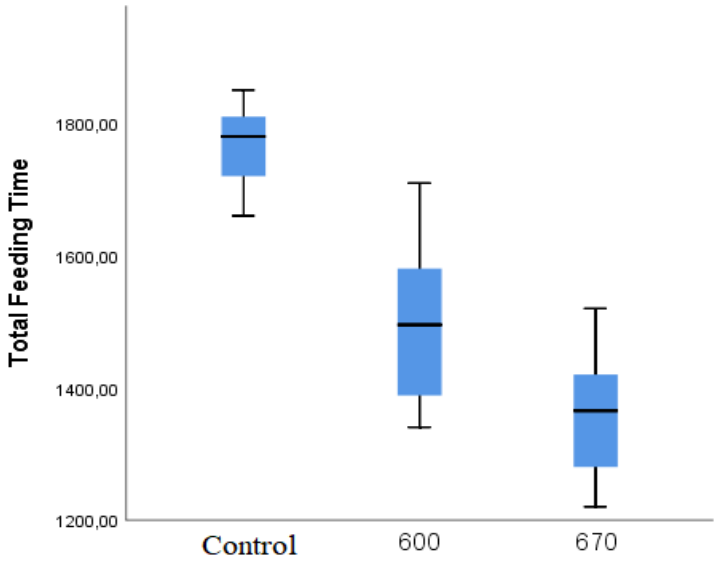

Figure 4. Distribution of total feeding times of *Nezara viridula* individuals exposed to doses of 600 and 670 ppm carbon dioxide and not treated with carbon dioxide (control).

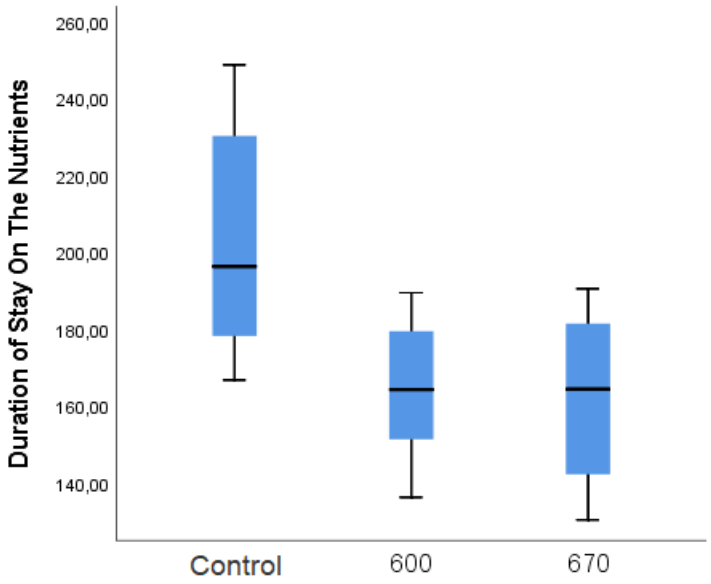

Figure 5. Distribution of duration of stay on the nutrients of *Nezara viridula* individuals exposed to doses of 600 and 670 ppm Carbon Dioxide and not treated with carbon dioxide (control).

When the pairwise comparisons are examined, it is seen that the duration of stay on the nutrients of the individuals exposed to 600 and 670 ppm application doses is significantly lower than the duration of stay on nutrients of the individuals in the control group ($p<0.05$) (Figure 5).

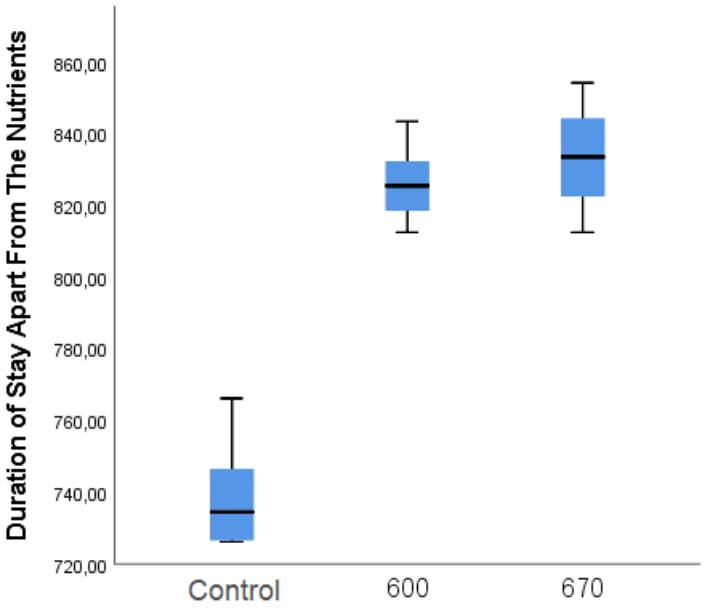

Figure 6. Distribution of the duration of stay apart from nutrients of *Nezara viridula* individuals exposed to 600 and 670 ppm doses of carbon dioxide and not treated with carbon dioxide (control).

Considering the pairwise comparisons, the duration of stay on the nutrients of the individuals to whom 600 and 670 ppm application doses were applied was found to be significantly higher than the duration of stay apart from the nutrients of the individuals in the control group ($p<0.05$) (Figure 6).

CONCLUSION

In a study conducted by Birgücü and Karsavuran (2013) on the nutritional behavior of *Nezara viridula* in bean pods, it was reported that the duration of stay on the nutrients of male individuals from the plant in their adult periods was lower than that of female individuals in the oviposition period, and the duration of stay on the plant was higher. However, they stated that the total feeding time of the pest was longer than the duration of stay apart from the nutrients and the duration of stay on the plant in all biological periods. In a study conducted by

Yiğit (1988) on the main nutritional characteristics of *Liorhyssus hyalinus* (F.) adults, it was reported that the active feeding time of male individuals in the hibiscus plant of the pest constituted19.39% of the observation time and female individuals constituted 18.97%. In addition, in the same study, it was stated that the observation period of the active nutrition of male and female individuals constituted 14.98% and 22.13%, respectively, on the wild lettuce plant.

In a study conducted on adult individuals of *Halyomorpha halys* Stål *(*Pentatomidae), *Riptortus clavatus* Thunberg (Alydidae), *Nezara antennata* Scott (Pentatomidae) and *Piezodorus hybneri* Gmelin (Pentatomidae) belonging to the Hemiptera team, the nutritional behaviors of the pests on the soybeans of male and female units were observed. According to the results of this study, in which the duration and frequency of daily feeding were examined separately for each species, adult female individuals of *H. halys* performed feeding activities 3.65 times a day. The pest was fed for 1.70 h in each feeding activity. The total daily feeding time of female individuals is 6.20 hours. Male individuals, on the other hand, performed feeding activities 2.71 times a day and showed nutritional behavior for 1.99 hours in each nutritional activity. The total daily feeding time of male individuals is 5.39 hours. Adult female individuals of *R. clavatus* performed feeding activities 2.65 times a day and the pest was fed for 2.02 h in each feeding activity. The total daily feeding time was determined as 5.36 hours. Male individuals, on the other hand, performed nutritional activities 4.00 times a day and performed nutritional behavior for 2.13 hours in each nutritional activity. The total daily feeding time of male individuals is 8.50 hours. Adult female individuals of *N. antenata* performed feeding activities 3.80 times a day and it was observed that the pest was fed for 2.97 hours in each feeding activity. The total daily feeding time was 11.29 hours. Male individuals, on the other hand, performed nutritional activities 4.00 times a day and the pest was fed for 4.04 hours in each nutritional activity. The total daily feeding time of male individuals is 16.17 hours. Adult female individuals of *P. hybneri* were fed 2.67 times a day for 3.94 hours in each feeding activity. The total daily feeding time is 10.52 hours. Male individuals, on the other hand, performed nutritional activities 4.50 times a day and the pest has engaged in nutritional behavior for 2.89 hours in each nutritional activity. The total daily feeding time of male individuals is 13.00 hours (Kawamoto et al., 1987).

According to the results of a study conducted by Birgücü and Karsavuran (2013) on the nutritional behavior of *N. viridula*, it was observed that the nutritional duration of the pest was 16.97 h when calculated from the total nutritional duration of adult male individuals within one hour of observation. Daily feeding times of female individuals in the preoviposition and oviposition period were determined as 16.46 h and 15.02 h, respectively. In this case, it is possible to say that adult male and female individuals of *N. viridula* feed more in a day than the pests in the study conducted by Kawamoto et al. (1987) and that adult individuals of *N. viridula* show more frequent feeding activities in a day. In addition, adult individuals of *N. viridula* showed a total feeding time of 1,924.92 seconds (0.53 h), with an initial period of 25.82 seconds (0.007 h), probing time of 32.54 seconds (0.009 h), and active feeding time of 1,866.59 seconds (0.51 h) in a single repetition. Accordingly, it can be said that adult individuals of *N. viridula* fed for less time at a single time than the pests in the study conducted by Kawamoto et al. (1987). All these results support the opinion that *N. viridula* is one of the most harmful species among pentatomids (Lodos et al., 1978).

As a general result of this study, it was determined that as the application dose increased, the nutritional behaviors of male and female individuals changed, male individuals reacted more to dose administration and remained on nutrients less than female individuals.

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The authors declare that they have no conflict of interest.

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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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Detection and monitoring of MC-LR and MC-RR in the artifical irrigation ponds at Oltu district

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Abstract
Although it is defining as natural organic pollutant, surface water resources which are frequently exposed to Harmful Algae Blooms (HABs) due to the increasing nutrient loading in recent years, the increase in temperature caused by climate change and the increase in surface run-off caused by extreme rainfall as a result of the risk of the increasing concentrations of algal toxins into drinking water. Although HABs have caused the problem of eutrophication in surface waters especially at hot seasons in the past decades due to the water pollution, increasing surface water temperatures with climate change cause this problem to extended periods out of season and to be permanent for the year. Therefore, studies including the detection and monitoring of algal toxins are gaining importance in order to observe HAB events at their source. As global temperature increases, HAB events have spread to regions that have even cold climates. Consequently, Microcystin-LR (MC-LR) and Microcystin-RR (MC-RR), which are the main indicators of HAB events in surface waters were aimed to detect and monitor at the artificial ponds designed for agriculture and animal husbandry purposes in Oltu District of Erzurum Province which has cold climate, for the first time in this study.Microcystins (MCs) concentrations were measured in the samples taken from ponds in four seasons for one year, by LC-MS/MS and; water temperature and pH values were also determined simultaneously. The relationship between the MC-LR and MC-RR distributions and, the pH and temperature were calculated by Pearson Correlation Coefficient (r).

Keywords: Harmful Algal Blooms, Eutrophication, Microcystins, Surface Water Pollution, Climate Change

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INTRODUCTION

In recent years, drinking water treatment plants (DWTPs) have focused on newly emerging micropollutants (MPs) in the water resources rather than classical pollutants. Since they are generally designed for basic parameters such as taste, odor, color, turbidity, dissolved oxygen, and therefore the treatment of MPs that emerge in the water environment in both type and quantity and cause concern for public health cannot be possible in traditional DWTPs. Among these MPs, some toxic compounds caused by HABs have become frequently researched groups due to their hazards to the aquatic ecosystem and public health (Simith et al., Turner et al. 2018; Munoz et al. 2021).

Since these compounds released from algal cells during HAB events cannot be treated in DWTPs, they may directly affect public health and may also be harmful to the aquatic ecosystem (Wu et al. 2011; LaLiberte and Haber, 2014). The main groups known to cause HABs and produce toxins in the freshwater environment are cyanobacteria, also known as blue-green algae. Microcystis group algae, which are the most important species known to produce toxins among cyanobacteria species, are responsible for global eutrophication and have become the biggest problem of DWTPs by causing taste and odor problems in drinking water (Vidal et al. 2021). WHO reports state that 60% of HABs event contain toxins and these toxins are generally called cyanobacterial toxins classified as MCs, cylindrospermopsins, anatoxins, saxitoxins, anatoxin-a (s) and dermatotoxins, which have

important effects on human health. These toxins can be produced by more than one species; for example, the MC group cyanobacterial toxin can be produced by any of the Microcystis, Planktothrix, Dolichospermum and Nostoc species. These species are known as the primary MCs producing species in freshwater environments. MCs are the largest and most diverse group of cyanotoxins, consisting of more than 80 species. The most common producers of MCs are known as Microcystis aeruginosa (Žegura et al. 2011; Mishra et al. 2018; Díez-Quijada et al. 2019). As one of the well-known and most researched compounds of MCs, MC-LR should not be exceed 1 µg/L recommended by the World Health Organization (WHO) due to its carcinogenic properties (WHO 1998). In 2010, the International Agency for Research on Cancer (IARC) classified MC-LR as a possible human carcinogen Group 2B (He et al. 2016; Chen et al. 2018). Cyanobacterial toxins are classified as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins/tumour-promoter and irritant toxins (lipopolysaccharides) according to their hazard mechanism on animals and plants (Du et al. 2019), as they have the potential to cause death in aquatic organisms, form tumors or affect nutrition, growth and the immune system (Zhang et al. 2019; Shi et al., 2021).

WHO has determined limit values for both cyanotoxins and cyanobacteria (WHO 2003) for the protection of freshwater resources against HAB events. For this purpose, if water media contains 20,000 cyanobacterial cells in 1 mL, it indicates a low probability of adverse health effects, if it is in between 20,000-100,000 cyanobacterial cells in 1 mL it indicates a moderate probability of harmful health effects, and finally if it is greater than 100,000 cyanobacterial cells in 1 mL then this is a high probability of harmful health effects and long-term effects in humans that will cause many diseases. In a study where cyanobacteria were monitored in Yerevan Lake in Armenia, $695.9x10^3$ cells in 1 mL were analyzed and it was determined that they were highly hazardous to public health (Minasyan et al. 2018). First outbreak was recorded at 1996 at a diyalysis center in Caruaru, Brazil. 130 patients had died of acute liver failure, where the center received water from a nearby reservoir and Microcystins produced by cyanobacteria were detected in the water supply (Pouria et al., 1998; Jochimsen et al. 1998). Later In 2013, MC amounts were detected in the range of 1.4 µg/L-3.6 µg/L in treated drinking water in Ohio (USA), and the autorities explicitly warned to public as "do not drink" fort he first time (He et al. 2016) considering the limit value set by WHO (2020).

The occurrence of toxic cyanobacteria in freshwater has been increasing in both frequency and distribution in recent years due to increased global temperatures as well as water water pollution (Eren,2021; Köker et al. 2021), because there is a scientific consensus that ecosystem impacts caused by HAB have increased especially in the last few decades. In addition, increasing water temperatures due to climate change may cause cyanobacterial growth to spread over wider seasons. As a matter of fact, it was stated for the first time that this frequency of HABs was directly linked to climate change in the “Ocean and Cryosphere in a Changing Climate (SROCC)” Special Report of the United Nations (UN) Intergovernmental Panel on Climate Change (IPCC) approved in September 2019 (IPCC 2019). For instance, Lake Taihu as China’s third largest lake is an important drinking water source for many major cities including more than 30 million people. In 2007, record temperatures in China caused dramatically HAB event, releasing of toxins into the drinking water (Wu et al. 2011). Recent studies have revealed that 54% of the lakes in the Asia Pacific Region, 53% in Europe, 28% in Africa, and 48% and 41% in North America and South America, respectively, are eutrophic associated with HABs, due to increasing surface water temperatures and pollution loads in aquatic environments (WHO 1998).

In acute contact with water sources containing cyanobacterial toxins may be caused abdominal pain, vomiting, diarrhea, irritation of the eye, nose and throat mucosa, asthma attacks, nausea, tingling in the fingertips and toes, blurred vision, headache, dizziness, fever, hypoxia, resulting in paralysis and even death (Pantelić et al. 2013). Other episodes of cyanobacteria-associated human diseases are included some examples of a statistically significant correlation between drinking water from reservoirs containing Microcystis aeruginosa in Australia and signs of liver damage (Metcalf and Codd, 2004) and a high incidence of primary liver cancer in China, attributed to drinking water contaminated with cyanobacterial toxins (Harada et al.1996).

HAB monitoring and detection studies have gained great importance worldwide. Especially the increased temperature by climate change have led to HAB events not only in hot climate regions but also in cold climate regions. Although Erzurum is a cold climate city but climate temperatures have been increasing in recent years, no monitoring studies have been conducted in this region in terms of algal toxins until now. Therefore, MCs anaylsis method was developed for two important algal toxins (MC-LR and MC-RR) considered as indicators of HAB events and their monitoring in surface water resources was carried out for the first time in this study. In order to contribute to the studies conducted in terms of the increase, distribution and spread of HAB events in our country, the first HAB monitoring study was conducted in our region for protecting water resources becoming increasingly important. This is the first step of the multibarrier approach required to reduce the risk of toxic cyanobacterial growth in water resources. Accordingly, the first priority step is to prevent the contamination of the water source and control processes at the source, then it is important to use treatment technologies that will minimize the release of these toxins and optimize these methods. Therefore, determining and monitoring the amounts of cyanobacterial toxins in water resources is the most important step in eutrophication control (He et al. 2016). For this purpose, MC-LR and MC-RR was aimed to detect and monitor in 8 artificial ponds created for agricultural irrigation and husbandary purposes within the borders of Oltu District of Erzurum City in this study.

Composite water samples were taken from the ponds during three seasons, starting from August 2022, January 2023 and May 2023. The temperature and pH of the ponds were also measured during sampling period and the realtion of these parameters with MCs were investigated as well.

MATERIALS AND METHODS

Study Area

In order to detect MC-LR and MC-RR, two important MC species that occur as a result of HABs in surface waters, composite water samples were taken during three seasons, starting from August 2022, January 2023 and May 2023 in the 8 artificial ponds located within the borders of Oltu District, Erzurum City and created for agricultural irrigation and livestock purposes. Figure 1 shows the pictures taken in various periods of these ponds namely; Çengelli-1, Çengelli-2, Güzelyayla, İnanmış, Subatık, Ünlükaya, Bahçecik and Çatalsöğüt Ponds, respectively.

Figure 1. The MCs detection studies on 8 irrigation ponds in Oltu District, Erzurum.

Method

MC-LR and MC-RR compounds were analyzed with an LC-MS/MS. Based on literature searches, MC-LR constitutes 46-99.8% of the total MCs observed in HABs event (Sharma et al. 2012), MC-LR and MC-RR compounds were primarily selected to detect and analsis in this study. Analysis of MC-LR and MC-RR compounds was carried out with an LC-MS/MS, since they are found at nano or micro gram levels in surface waters. The LC-MS/MS device is located at Atatürk University Eastern Anatolia High Technology Application and Research Center (DAYTAM) and has an Agilent 6460 Triple Quadropol model system with Agilent G4226A autosampler and Jet Stream electrospray source. The LC-MS/MS device is created by combining liquid chromatography and spectroscopy systems that can provide robust, accurate qualitative and quantitative analysis of target compounds at extremely low levels. In order to perform analysis on the device, method validation was first carried out. Validation parameters for MC-LR and MC-RR compounds considering the literature studies; were determined as precision, accuracy, sensitivity, specificity and linearity. For method validation, samples were analyzed in 6 replicates (n>5). The LOD value was determined for a signal/noise ratio of 3. The LOQ value is determined as the value at which the signal/noise ratio is 10. The calibration curve was drawn to include the LOQ value in the range where the curve is linear (0.05, 0.10, 0.25, 0.5, 1, 5 and 10 µg/L) (Vashist and Luong, 2018). System suitability was achieved by 6 repeated injections of standard MC-RR at a concentration of 0.75 µg/L and standard MC-LR solution at a concentration of 1 µg/L (Pekar et al. 2016; Beversdrof et al. 2018; Aparicio-Muriana et al. 2022). The samples to be analyzed were taken as 10 mL, filtered through a 0.22 µm cellulosic filter and transferred directly to 2 mL autosampler vials (Figure 2). Table 1 includes the device operating conditions.

Table 1. LC-MS/MS Conditions

Colon	Agilent InfinityLab Poroshell SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)	
Colon Temp.	50 ºC	
Enjeksiyon hacmi	20 µL	
Mobile phase	A) 1 mM ammonium fluoride (HPLC grade) + %0.1 formic acid (%100 su V.03)	
A (%80) + B (%20)	B) 20% IPA + %0.1 formic acid (LC/MS grade) (%100 Acetonitryl V.03)	
Auto sampler temp.	5 ºC	
Flow rate	0.6 mL/min	
Gradient	Time, min	B, %
	3.00	30
	5.00	50
	6.00	100
Triple quadrupole MS Koşulları		
Ionization mode	ESI with Agilent Jet Stream Technology	
Drying gas temp. °C	350	
Drying gas flow, L/min	12	
Nebulizer pressure	40 psig	
Sheath gas temp.	400 °C	
Sheath gas flow	11 L/min	
Capillary voltage	4,000 V	
Nozzle voltage	1,000 V	
EMV	400 V	

Figure 2. The water samples to be analyzed in LC-MS/MS

RESULTS AND DISCUSSION

The concentrations of MC-LR and MC-RR in the water samples taken 3 times from each irrigation pond were analyzed 3 times on the LC-MS/MS and the results were obtained as in Figures 3-4 to better understand the MC-LR and MR-RR occurance during three seasons. Each season were selected to monitor the HAB event for the representative of summer, winter and spring on August 2022, January 2023 and May 2023, respectively. The samples collected from the ponds were stored at +4 ºC at dark conatiner and delivered to the laboratory, made ready for analysis and analyzed within 48 hours. The temperature of ponds and pH were also analyzed during the sampling time.

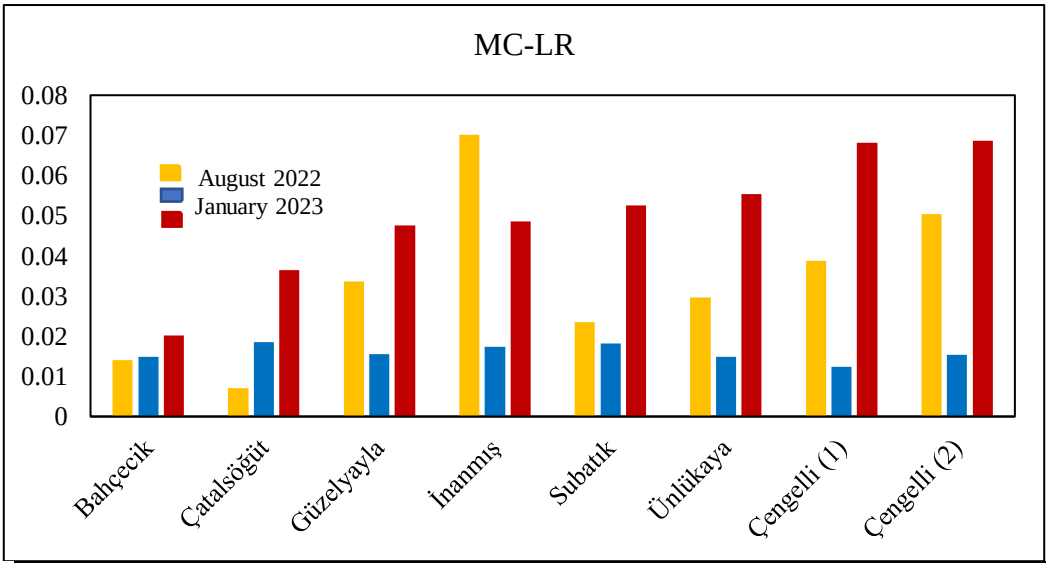

Figure 3. The Comparision of MC-LR concentration of ponds.

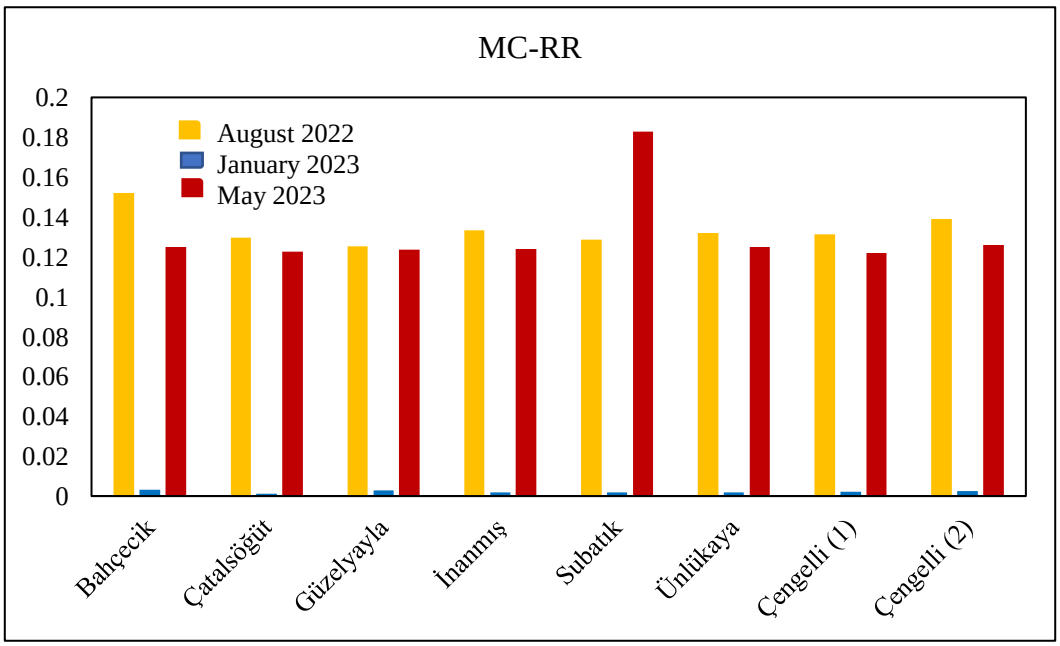

Figure 4. The Comparision of MC-RR concentration of ponds.

It can be evaluated that MC-LR and MC-RR concentrations in artificial irrigation ponds have reached visible levels from Figure 4 and Figure 5. MC-LR concentrations were detected in the range of 0.0071-0.0701 µg/L, while the concentration of MC-RR was analyzed in the range of 0.1253-0.1522 µg/L, during first anaylsis period of August 2022. Considering the 1 µg/L limit value determined by WHO for MC-LR, the ponds monitored in this study appear to pose a low risk for humans and animals which are drinking water from those areas. However, it is important to monitor MC concentrations in different seasons and long periods for the observation of HAB events.

For winter season, the amount of MC-LR in the ponds was analyzed in the range of 0.0124-0.0185 µg/L and MC-RR was analyzed in the range of 0.0013-0.0033 µg/L. While MC-RR concentrations were observed to decrease, MC-LR was observed to increase compared to the summer period that may indicate a possible accumulation due to their resistance to degradation. Finally, MC-LR and MC-RR concentration were analyzed in the ranges of 0.0202-0.0687 µg/L and 0.1251-01827 µg/L during spring season of May 2023.

When comparing the literature studies with our results; the concentration of MC-LR dissolved in water was 0.04 µg/L and the concentration of MC-RR was 1.50 µg/L were detected in Los Padres Lake in Argentina in 2007 (Amé et al. 2010). In a study conducted in Yanghe Reservoir in China in 2010, dissolved MC-RR was determined

as 1.56 µg/L, while MC-LR was determined as 0.544 µg/L (Li et al. 2010). Analyzes conducted in 14 regions in Northern Ireland show that MC-LR varies between 0.004–0.014 µg/L and MC-RR varies between 0.005–0.060 µg/L (Mooney et al. 2011). Analyzes conducted in Europe in the Jeziorsko reservoir in Poland found as 0.38 µg/L for MC-LR and 0.23 µg/L for MC-RR (Mankiewicz-Boczek et al. 2011). In analyzes conducted in clean water sources in Uganda, MC-LR was found to be 3.3 and MC-RR was found to be 23.0, indicating major contamination (Okello et al. 2010). In a study conducted in two different water reservoirs in Portugal, MC-LR was detected in the range of 15–344 ng/L and MC-RR 14–212 ng/L (Rodrigues et al. 2013). As a result of the analysis made on samples taken from water bodies in six different countries (France, Italy, Germany, Bulgaria, Ireland), including Turkey, MC-LR was found to be in the range of 0.1–132.8 µg/L and MC-RR was 0.07–103.2 µg/L (Pavlova et al., 2015).

Pearson correlation measures the strength of the linear relationship between two variables. It has a value between -1 and 1, - value means total negative linear correlation, while + value means total positive correlation. 0 means no correlation (Berman 2016). Additionally, $0< r \leq 0.19$ suggests very low correlation; $0.2 \leq r \leq 0.39$ is low correlation; $0.4 \leq r \leq 0.59$ is moderate correlation; $0.6 \leq r \leq 0.79$ is high correlation and $0.8 \leq r \leq 1.0$ is very high correlation (Selvanathan et al. 2022). For this purpose, the relationship between the measured MC-LR and MC-RR concentrations and, the pH of the ponds and water temperature of the ponds was investigated with Pearson Correlation and, the Pearson Correlation Coefficient (r) values were calculated and shown in Tables 2-4 with rRR for MC-RR and rLR for MC-LR, together with the total measurement parameters. The pH value of the ponds was between 8.2-9.7; the temperatures vary between 18.1-18.4 ºC during August 2022 season, water temperatures in the ponds were measured in the range of 0.7-2.4 ºC and pH in the range of 8.4-9.5 for winter 2023 season and, finally pond temperatures varied between 12.3-21 ºC, pH varied between 6.1-8.9 and DO values varied between 5.1-8 mg/L during spring 2023 season.

Table 2. Detection of MC-LR and MC-RR concentrations in ponds at August 2022.

	Pond	**pH**	**Temp. ºC**	**MC-LR µg/L**	**MC-RR µg/L**	**r**
1	Bahçecik	8.3	18.2	0.0141	0.1522	
2	Çatalsöğüt	8.2	18.3	0.0071	0.1298	
3	Güzelyayla	9.4	18.1	0.0336	0.1253	
4	İnanmış	9.3	18.4	0.0701	0.1333	
5	Subatık	8.6	18.4	0.0235	0.1287	
6	Ünlükaya	9.1	18.2	0.0296	0.1319	
7	Çengelli (1)	9.4	18.1	0.0388	0.1312	
8	Çengelli (2)	9.7	18.0	0.0504	0.1390	
		0.80 (P<0.05)	-0.06			r_{LR}
		-0.27	-0.17			r_{RR}

Table 3. Monitoring of MC-LR and MC-RR concentrations in ponds at January 2023.

	Pond	**pH**	**Temp. ºC**	**MC-LR µg/L**	**MC-RR µg/L**	**r**
1	Bahçecik	8.9	2.4	0.0149	0.0033	
2	Çatalsöğüt	8.4	1	0.0185	0.0013	
3	Güzelyayla	9.4	0.7	0.0155	0.0027	
4	İnanmış	9	1	0.0174	0.0017	
5	Subatık	8.4	7.5	0.0182	0.0019	
6	Ünlükaya	9	2.3	0.0148	0.0020	
7	Çengelli (1)	9.5	2.3	0.0124	0.0021	
8	Çengelli (2)	9.5	1	0.0154	0.0024	
		-0.79 (P<0.05)	0.25			r_{LR}
		0.45	-0.07			r_{RR}

Table 4. Monitoring of MC-LR and MC-RR concentrations in ponds at May 2023.

	Pond	pH	Temp. °C	MC-LR µg/L	MC-RR µg/L	r
1	Bahçecik	8.9	15.2	0.0526	0.1827	
2	Çatalsöğüt	7.45	20.7	0.0687	0.1260	
3	Güzelyayla	8.9	21	0.0364	0.1226	
4	İnanmış	7.43	15.1	0.0553	0.1249	
5	Subatık	7.45	20.2	0.0202	0.1251	
6	Ünlükaya	7.2	20	0.0486	0.1239	
7	Çengelli (1)	8.5	12.3	0.0475	0.1235	
8	Çengelli (2)	6.12	21	0.0682	0.1220	
		-0.39	-0.03			r_{LR}
		0.49	-0.36			r_{RR}

According to the results shown in Tables 2-4, although the significance levels could not be determined at a sufficient level since the number of data was small, a high significance level relationship (0.80) was detected between water pH and MC-LR concentration in samples taken during August 2022 ($P<0.05$). It is thought that MC-LR concentrations in water are responsible for the pH changes in the summer period. A similar situation was detected in samples taken in January 2023, which is the winter period. Accordingly, a high significance level negative relationship (-0.79) was detected between water pH and MC-LR concentration ($P<0.05$). MC-LR concentration decreases in January 2023 negatively affected pH changes. When the relationship of MC-RR with pH in ponds was examined; a low negative relationship (r_{RR}=-0.27) was determined in August 2022, and a moderate positive relationship was determined in January 2023 and May 2023 (r_{RR} values 0.45 and 0.49, respectively). Finally, a low relationship was observed between water temperature and MC concentrations in samples taken in each period.

CONCLUSION

In this study, eight irrigation ponds in Oltu District of Erzurum City were selected to detect and monitor of MC-RR and MC-LR during summer, winter and spring seasons 2022-2023. Although MCs were not found in high concentrations based on WHO limits in the pons, the indication of HAB events can cause a significant threat to both the environment and public health. Therefore, risk analysis and management tools as well as sustainable and cost-effective treatment processes need to be developed. The presence of cyanotoxins in freshwater is confirmed in many countries around the world, but limited information is still available and further monitoring is required. In addition, monitoring, analysis, toxicology, treatment methods are carried out only on certain types of MCs. In addition, it is also necessary to develop preventive strategies such as preventing pollution in irrigation water supply systems, especially for drinking and use or agriculture-livestock purposes, developing strategies to reduce nutrient loads, and early detection of algal blooms with remote sensing systems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Mevra Emeç contributed to Sample Collection, Data Obtaining, Writing—Review & Editing.

Zeynep Eren contributed to Conceptualization, Methodology, Data analysis, Visualization, Writing—Original Draft, and Review & Editing.

Data availability

Data will be made availabale on request.

Consent to participate

The authors consent to participate.

Consent for publication

The authors consent to publication.

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Comparative analysis of raw milk samples in Amasya region

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DergiPark AKADEMİK

Abstract
Milk is a complete and incredibly nutritious food supply for humans. Millions of tons of raw milk have been processed by the dairy industry to meet huge public demand. Therefore, there are studies needed related to the classification of raw milks in the supply chain. The aim of this study is to evaluate the quality of raw milk samples in Amasya region. Firstly, raw cow's milk is classified according to its protein and fat values. In the first period, the mean fat value of raw milk collected from three lines was found to be 3.86, 3.89, and 3.87 as (%) the percent value, respectively, while the mean protein value of raw milk collected from three lines was found to be 3.29, 3.28, and 3.25 as (%) the percent value, respectively. In the second period, the mean fat value of raw milk collected from three lines was found to be 3.93, 3.99, and 4.03 as (%) the percent value, respectively, while the mean protein value of raw milk collected from three lines was found to be 3.34, 3.35, and 3.34 as (%) the percent value, respectively. The findings indicated that during both periods, the daily raw milk collected from three lines is class A, where protein value (%) is 3.1 and above while fat value (%) is 3.5 and above. Since the quality of raw milk is important not only for milk consumers but also for the quality of the corresponding dairy products, the quality of raw milk must be controlled correctly. Consumer requirements for high-quality milk and dairy are of importance on dairy products.

Keywords: Milk Classification, Statistical Analysis, ANOVA, Dairy Industry

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INTRODUCTION

The dairy industry is an important component of many economies and is an important industry in most developed and developing economies of the world. The dairy industry is a large-scale industry due to the large number of farms, collection centers, production facilities, and distribution centers. Dairy products are sensitive to environmental conditions and are affected by rapid changes in environmental conditions (Sel and Bilgen, 2015). Dairy industry is an important income source in Türkiye. In Türkiye, raw milk production was 21 million 563 thousand 492 tons in 2022, decreased by 0.4% in 2023, and became 21 million 481 thousand 567 tons (TÜİK, 2023). To improve the quality of dairy products, it is important to use raw milk of appropriate quality. Collecting different types of milk entails significant additional logistics costs in an uncertain environment. Therefore, it is very important to organize a healthy distribution system to control the supply chain for dairy products. In particular, the distribution of milk from farms to consumers can pose high risks in terms of pathogens and nutritional losses. Milk, unlike other products, needs a protection and monitoring system to ensure healthy conditions (Sayin et al., 2011). Demirbaş et al. (2009) examined the role of milk collection centers in supplying quality and safe milk. They determined that milk collection centers, which act as bridges between farmers and the dairy factory, have an important role in food safety and quality assurance in the milk processing industry. Mu et al. (2016) examined the effect of supply chain management on the quality of milk in their study. In order to reduce testing costs in their study, they proposed mixed testing, in which milk from more than one farmer is tested once. Sayin et al. (2011) used an empirical (Logit) model to examine the factors affecting the role of milk collection centers in providing a safe distribution channel in Türkiye. According to the results, milk sales decisions are significantly affected by income and demographic characteristics. Ahmad et al. (2010) used multiple linear regression, artificial neural network, and k-nearest neighbor techniques to predict raw milk quality. Sangatash et al. (2012) used fuzzy logic

in their study to classify raw milk according to microbiological and physicochemical qualities. Berhilevych et al. (2019) estimated the number of bacteria from the *Enterobacteriaceae* family in raw milk stored under refrigeration conditions using an artificial neural network. Reguillo et al. (2018) analyzed the effect of milk collection frequency and milking time on the numbers of total mesophilic aerobic bacteria and psychrotrophic bacteria in raw milk samples. Milking in the morning showed a significant decrease in the number of mesophilic aerobic bacteria compared to milking in the evening. Ndahetuye et al. (2020) analyzed the effects of somatic cell count, total bacterial count, *Escherichia coli*, *Salmonella*, and *Brucella* species on the quality of milk. They determined that contamination of milk with *Escherichia coli* occurred more frequently in milk collection centers. Preka and Bekteshi (2016) presented physicochemical characteristics of cow's milk gathered in the Shkodra region. In their study, Uzun and Allahverdi (2021) developed a fuzzy logic-based decision support system to predict the spoilage of heat-treated raw milk. In Tohidi et al. (2018), they detected the presence of detergent powder in raw milk with an artificial olfactory machine based on eight metal oxide semiconductor sensors. Multivariate analysis of variance was used to optimize the data matrix. Additionally, linear discriminant analysis, support vector machine, and adaptive network-based fuzzy inference system method were used for qualitative classification. Cebeci (2019) determined the microbiological quality of raw cow milk samples sold in public markets and investigated foodborne pathogens in Giresun. In their study, Neware (2023) checked the quality of cow milk with a data set consisting of 1059 milk samples taken from various cows using k-nearest neighbor, logistic regression, support vector machine and artificial neural network.

Figure 1. The map of Amasya province (Anonymous, 2024).

To summarize, since the quality of raw milk is important not only for milk consumers but also for the quality of related dairy products, the quality of raw milk must be accurately controlled. Each milk delivery is inspected to determine certain qualitative characteristics. Microbiological and physicochemical analyses are important in monitoring the quality of raw milk and its products. Consumer requirements for high-quality milk and dairy products have placed a significant responsibility on dairy producers, so dairy factories need a good system to evaluate and classify the quality of the raw milk they receive (Sangatash et al., 2012). Studies in the literature are generally developed for specific problems. Models with problem-specific restrictions and assumptions are not suitable for every type of problem. Therefore, there is a need for researchers to design improved methods that can be used to evaluate the quality of raw milk. The objective of this study is to provide an academic contribution by utilizing statistical analysis to assess the quality of raw milk in Amasya region.

MATERIALS AND METHODS

Proposed Methodology

Dataset is provided from the Cattle Breeders' Association of Amasya. Association was founded in 1998 by dairy cattle breeders operating in the Amasya region (Figure 1). Every day, samples taken from milk cooling tanks, milk collection vehicles, and factory milk purchasing vehicles in the villages are analyzed (AIDSYB, 2024). Kjeldahl analysis method was used to determine protein content of milk samples. Gerber method was employed for fat content determination. Precise weighing method using laboratory style oven was employed for the determination of dry matter value. The dataset was first evaluated according to fat and protein values. Raw cow milk is classified according to its protein and fat values as shown in the table below (Anonymous, 2019).

Table 1. Raw cow milk classification (Anonymous, 2019).

Class	Protein Value (%)		Fat Value (%)	
	October – March (Second period)	April – September (First period)	October – March (Second period)	April – September (First period)
Class A	3.2 and above	3.1 and above	3.6 and above	3.5 and above
Class B	$3 \leq protein\ value < 3.2$	$3 \leq protein\ value < 3.1$	$3.3 \leq fat\ value < 3.6$	$3.2 \leq fat\ value < 3.5$
Class C	$2.9 \leq protein\ value < 3$	$2.9 \leq protein\ value < 3$	$fat\ value < 3.3$	$fat\ value < 3.2$

In this paper, three different lines (village or individual) that arrived at the milk collection center were examined. The first line (Line 1) includes Balgöze, Ortaova, Gümüştepe, Oymaağaç, Yolüstü, Sarıbuğday, Karşıyaka, Yalnız, Çaybaşı, Mahmutlu, individual 2, individual 5, and individual 6. Second line (Line 2) includes Sarıköy, Alıcık, Karamağara, Elmayolu, Diphacı, Hanköy, Doluca, Akören, Bulak, individual 1, and individual 4. Third line (Line 3) includes Hacıyakup, Yakup, Kıreymir, Yakacık, Sazlıca, and Pekmezci. Descriptive statistics for the first period and second period are given in Table 2 and Table 3 for three lines. According to the results, all lines are Class A.

Table 2. Descriptive statistics for first period.

		N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
Fat value	Line 1	145	3.86	0.14	0.01198	3.54	4.21
	Line 2	145	3.89	0.11	0.00939	3.62	4.21
	Line 3	145	3.87	0.16	0.01295	3.55	4.42
	Total	435	3.87	0.14	0.00666	3.54	4.42
Dry matter value	Line 1	145	8.86	0.14	0.01137	8.52	9.18
	Line 2	145	8.86	0.12	0.01002	8.58	9.19
	Line 3	145	8.80	0.11	0.00895	8.56	9.08
	Total	435	8.84	0.13	0.00600	8.52	9.19
Protein value	Line 1	145	3.29	0.06	0.00509	3.14	3.42
	Line 2	145	3.28	0.05	0.00404	3.16	3.40
	Line 3	145	3.25	0.06	0.00485	3.13	3.38
	Total	435	3.27	0.06	0.00278	3.13	3.42

Table 3. Descriptive statistics for second period.

		N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
Fat value	Line 1	153	3.93	0.10	0.00773	3.72	4.16
	Line 2	153	3.99	0.09	0.00698	3.77	4.20
	Line 3	153	4.03	0.17	0.01347	3.73	4.59
	Total	459	3.98	0.13	0.00602	3.72	4.59
Dry matter value	Line 1	153	8.90	0.14	0.01169	8.64	9.19
	Line 2	153	8.95	0.07	0.00557	8.80	9.14
	Line 3	153	8.91	0.11	0.00862	8.71	9.17
	Total	459	8.92	0.11	0.00528	8.64	9.19
Protein value	Line 1	153	3.34	0.04	0.00311	3.27	3.43
	Line 2	153	3.35	0.04	0.00293	3.27	3.43
	Line 3	153	3.34	0.03	0.00257	3.26	3.43
	Total	459	3.35	0.04	0.00169	3.26	3.43

Analysis of variance (ANOVA) was used to analyze whether there were differences between lines. ANOVA is a method used to test the difference in group means or treatment means of data obtained from k independent or k dependent groups (Özdamar, 2013). The following hypothesis was tested in this study.

H_0: There is no difference between the means.

H_1: At least one mean differs from the others.

In this paper, Welch's ANOVA is used. Welch's ANOVA is a test for multiple comparisons of means. It is a modified One-Way ANOVA that is robust to the assumption of equal variances. Welch's ANOVA is an extension of the 2 sample t-test for means, assuming unequal variance (Sigmaxl, 2024).

RESULTS AND DISCUSSION

Milk is an important source of protein, including casein and essential amino acids, fats, and total solids that define milk's quality from an industrial perspective. Fat content is crucial because yield of butter production or production of dairy products with high fat content matters in industrial processing of milk. Protein and total solid content are important not only in terms of nutritional value but also structural quality of dairy products (Guetouache et al., 2014). Confidence interval plot for samples are given in Figure 2-7.

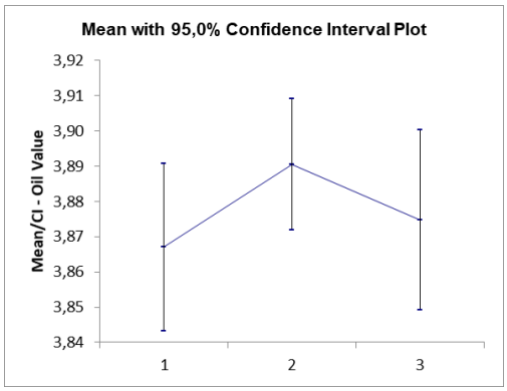

Figure 2. Confidence interval plot for fat value in first period.

For fat value in the first period, the group means are equal because the p-value for Welch's ANOVA is 0.2754, which suggests that we do not reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 4.

Table 4. Pairwise mean difference and Welch pairwise probabilities for fat value in first period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	-0.02	-0.01
2		0	0.016
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		0.12	0.66
2			0.32
3			

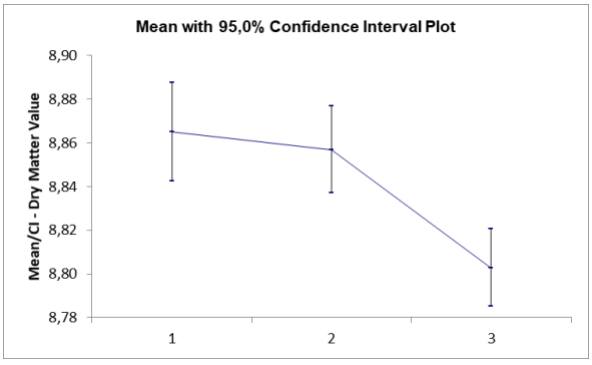

Figure 3. Confidence interval plot for dry matter value in first period.

For dry matter value in the first period, the group means are not equal because the p-value for Welch's ANOVA is 0, which suggests that we reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 5. It can be concluded that the dry matter value in the first period is different between Line 1 and 3. In addition, the dry matter value in the first period is different between Line 2 and 3.

Table 5. Pairwise mean difference and Welch pairwise probabilities for dry matter value in first period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	0.01	0.06
2		0	0.05
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		0.59	**0.00**
2			**0.00**
3			

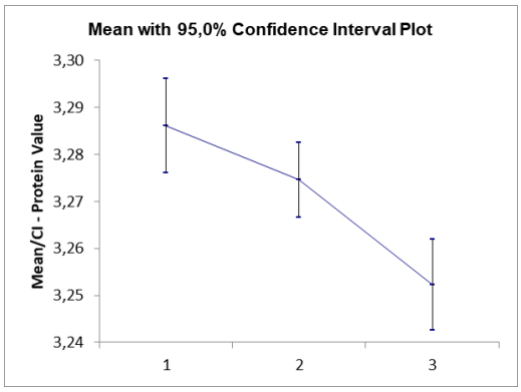

Figure 4. Confidence interval plot for protein value in first period.

For protein value in the first period, the group means are not equal because the p-value for Welch's ANOVA is 0, which suggests that we reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 6. It can be concluded that the protein value in the first period is different between Line 1 and 3. In addition, the protein value in the first period is different between Line 2 and 3.

Table 6. Pairwise mean difference and Welch pairwise probabilities for protein value in first period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	0.01	0.03
2		0	0.02
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		0.08	**0.00**
2			**0.00**
3			

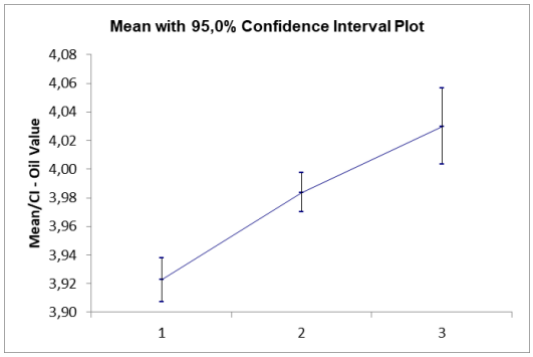

Figure 5. Confidence interval plot for fat value in second period.

For fat value in the second period, the group means are not equal because the p-value for Welch's ANOVA is 0, which suggests that we reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 7. It can be concluded that the fat value in the second period is different between Line 1 and 2. In addition, the fat value in the second period is different between Line 1 and 3. Also, the fat value in the second period is different between Line 2 and 3.

Table 7. Pairwise mean difference and Welch pairwise probabilities for fat value in second period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	-0.06	-0.11
2		0	-0.05
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		**0.00**	**0.00**
2			**0.00**
3			

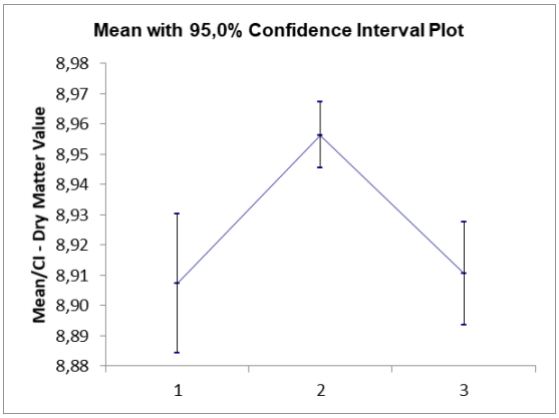

Figure 6. Confidence interval plot for dry matter value in second period.

For dry matter value in the second period, the group means are not equal because the p-value for Welch's ANOVA is 0, which suggests that we reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 8. It can be concluded that the dry matter value in the second period is different between Line 1 and 2. Also, the dry matter value in the second period is different between Line 2 and 3.

Table 8. Pairwise mean difference and Welch pairwise probabilities for dry matter value in second period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	-0.05	-0.00
2		0	0.05
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		**0.00**	0.82
2			**0.00**
3			

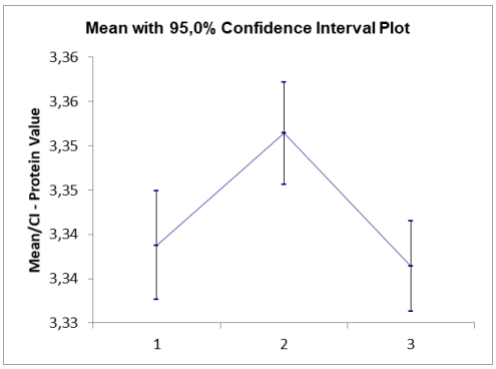

Figure 7. Confidence interval plot for protein value in second period.

For protein value in the second period, the group means are not equal because the p-value for Welch's ANOVA is 0.0004, which suggests that we reject H_0. Pairwise mean difference and Welch pairwise probabilities are given in Table 9. It can be concluded that protein value in the second period is different between Line 1 and 2. Also, protein value in second period is different between Line 2 and 3.

Table 9. Pairwise mean difference and Welch pairwise probabilities for protein value in second period.

Pairwise Mean Difference (row - column)	**1**	**2**	**3**
1	0	-0.01	0.00
2		0	0.01
3			0
Welch Pairwise Probabilities	**1**	**2**	**3**
1		**0.00**	0.55
2			**0.00**
3			

CONCLUSION

In conclusion, the mean fat value of raw milk collected from three lines was found to be 3.86, 3.89, and 3.87 as (%) the percent value, respectively, while the mean protein value of raw milk collected from three lines was found to be 3.29, 3.28, and 3.25 as (%) the percent value, respectively in the first period. In the second period, the mean fat value of raw milk collected from three lines was found to be 3.93, 3.99, and 4.03 as (%) the percent value, respectively, while the mean protein value of raw milk collected from three lines was found to be 3.34, 3.35, and 3.34 as (%) the percent value, respectively. The results showed daily raw milk collected from three lines is class A for both periods. Second, daily raw milk collected from three lines was compared considering fat, dry matter, and protein values. ANOVA was used for statistical analysis. Differences were found in group means for daily raw milk collected from three lines. This study shows that ANOVA analysis can be successfully implemented in classifying raw milk during the collection stage, and it may help accelerate milk selection in industrial applications in dairy processing. Classification is of importance in terms of hastening the processing of raw milk in the production of high quality dairy products. Therefore, this study sheds a light on using ANOVA to classify raw milk at the beginning of milk processing. In the future, more studies should be implemented on a wide range of areas to implement ANOVA to classify raw milk for huge dairy industries.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

SLİ designed, analyzed the data, and wrote the paper. The author read and approved the final manuscript.

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Determination of the effects of drought stress on *Aronia melanocarpa* cv. Nero *in vitro* conditions

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Abstract
Drought stress is a significant threat to plant cultivation in arid and semi-arid regions, negatively affecting growth and leading to plant mortality. This study evaluated the *in vitro* drought tolerance of *Aronia melanocarpa* 'Nero' by exposing micropropagated plantlets to nutrient media containing different concentrations of PEG-8000 (0%, 1%, and 2%) during the rooting stage. Results showed that increasing PEG concentrations led to a reduction in survival, rooting, plantlet growth, and physiological parameters. The 0% PEG treatment resulted in the highest survival rate (95.83%), root number, and chlorophyll content, while the 2% PEG treatment significantly hindered these parameters. These findings indicate that *Aronia melanocarpa* 'Nero' is sensitive to drought stress, with reduced growth and physiological activity under higher PEG concentrations.
Keywords: Chlorophyll, Drought stress, *In vitro*, Micropropagation, Growth

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INTRODUCTION

Aronia is a berry like fruit found within the *Aronia* genus of the *Rosaceae* family. This fruit is native to North America and spread to Europe at the beginning of the twentieth century. There are many varieties of aronia. However, in our country, the 'Viking' and 'Nero' varieties are at the forefront. Fresh aronia fruits are rarely consumed due to their bitter taste. Its fruits are mostly used in the production of fruit juice, wine, tea, medicine and nutritional supplements. In addition, the anthocyanins in its content can be used as natural food coloring. It is an important fruit for human health with its high antioxidant content. Its use against cancer has become intense due to its high nutritional values and the natural content of polyphenolic substances that fight against free radicals (Kapci et al., 2013; Vagiri and Jensen, 2017; Sidor and Gramza-Michałowska, 2019; Yilmaz et al., 2021; Zhang et al., 2021).

Aronia is a hardy species that can be grown in areas where winter temperatures drop to -30-35°C, as well as being tolerant to frost. Production and fruit quality are higher in areas that can receive sunlight. It is not selective in terms of climate characteristics. However, they are sensitive to drought conditions. When drought conditions continue, they cannot withstand long periods and the quality of the fruits decreases (Negreanu-Pirjol et al., 2023).

Drought stress is one of the abiotic stress factors and negatively affects production, product yield and quality. Exposure to drought stress during the plant's growth process restricts growth and development. Drought stress can affect the morphological parameters of the plant as well as inhibit the functions of physiological parameters such as stomatal movements, photosynthesis and respiration. Plants primarily respond to changes in their external and internal structures under drought stress conditions. As the severity of drought stress increases, it can result in slow plant growth and plant death in the future (Mahajan and Tuteja, 2005; Yang et al., 2021).

Tissue culture methods are preferred *in vitro* due to the sustainability of the studies under controlled conditions and regardless of the vegetation period (Mese and Tangolar, 2019). Some chemical substances are used to determine tolerance to drought stress *in vitro*. Drought stress occurs by adding these stress agents to culture media at certain rates. Agents that cause drought stress *in vitro* are sorbitol, mannitol and polyethylene glycol (Simsek et al., 2018; Sattar et al., 2021; Bilir Ekbic et al., 2022).

The agent commonly used in tissue culture studies is polyethylene glycol. Polyethylene glycol (PEG) is a substance that can imitate drought stress in the early stages of plant growth. PEG has forms with a wide molecular weight between 200-10.000.000 g/mol. PEG is a substance with an osmotic structure. It reduces the water potential in the environment in which it is used. It is not a toxic substance. It is used as an artificial abiotic stress stimulant and is included in many stress studies (Toosi et al., 2014; Gullapalli and Mazzitelli, 2015; Herzberger et al., 2016; Othmani et al., 2021; Violita and Azhari, 2021; Pham Le Khanh et al., 2022).

There are no sufficient studies in the current literature on the resistance of 'Nero' variety to PEG-induced drought stress, so this research aims to fill the knowledge gap on this subject. Therefore, this study was conducted to investigate the effects of PEG-8000-induced drought stress on the morphological and physiological parameters of the plant *in vitro*.

MATERIALS AND METHODS

The study was conducted in the plant tissue culture laboratory of the Horticulture Department of the Faculty of Agriculture, Harran University. Shoots taken from 1-year-old plants grown in greenhouse conditions during the active development period were used. Micro cuttings with a single node belonging to the 'Nero' variety of *Aronia melanocarpa* [Michx.] Elliot were used as plant material. The micro cuttings were first subjected to a pre-sterilization process in the laboratory. In the pre-sterilization process, the micro cuttings were kept in detergent water for 10 minutes and then rinsed under tap water until completely cleaned. After this, the micro cuttings were taken into a sterile cabinet and first kept in 70% ethyl alcohol for 2 minutes, then in 10% NaCIO (sodium hypochlorite) solution for 10 minutes and then rinsed 3 times with sterile pure (Nas et al., 2023). After this, micro cuttings were transferred to tubes containing 20 ml of growth medium containing 3% sucrose, 1 mg L^{-1} GA_3, 0.8 g L^{-1} agar and MS.The pH of the nutrient medium was adjusted to 5.8 with 1N NaOH and 1N HCI. The plantlets obtained in the shoot propagation medium were transferred to the tillering medium containing 3% sucrose, 3 mg L^{-1} BAP, 1 mg L^{-1} kinetin, 1 ml L^{-1} PPM and 7 g L^{-1} agar (Almokar and Pirlak, 2018). When the sufficient number of plantlets was reached, the trial was designed. The trial was established at the rooting stage of the plantlets. In the study, three different doses of PEG-8000 were added to the rooting medium (Table 1). 3% sucrose, 7 g L^{-1} agar, 2 mg L^{-1} IBA and 0.5 mg L^{-1} NAA were added to the rooting medium (Polat and Eskimez, 2022). %0, %1 and 2% PEG-8000 was added to the nutrient medium. Only rooting hormone was added to the control group. 1 ml L^{-1} PPM (Plant preservative mixture) was added to prevent bacterial contamination that may occur in the nutrient medium (Babu et al., 2022; Kara et al., 2022; Ekinci et al., 2024). The plantlets transferred to the applications were kept in a growth chamber using white fluorescent lamps, at 16:8 hours photoperiod, 65-70% air relative humidity and 25±1°C temperature for 60 days (Saskin et al., 2022). The study was designed according to a randomized trial design with 3 replications and 10 plants in each replication.

Table 1. PEG-8000 concentrations in culture media.

Treatments	
T1	%0 PEG-8000
T2	%1 PEG-8000
T3	%2 PEG-8000

Figure 1. Development of plantlets exposed to PEG-8000-induced drought stress *in vitro*. T1: 0% PEG-8000, T2: 1% PEG-8000, T3: 2% PEG-8000

Measurements and Statistical Analysis

The study was terminated after 60 days. At the end of the application, morphological and physiological parameters such as survival rate, rooting rate, number of root, root length, shoot length, number of leaves, plant

fresh weight, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll were examined. The data obtained at the end of the applications in the study were compared with the LSD test at the $p \leq 0.05$ significance level using one-way variance analysis in the JMP Pro 13 statistical program (Gomez and Gomez., 1984). Hierarchical clustering analysis (HCA) was conducted via the Software R (Version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Drought occurs due to global climate change and disruption of ecological balance. Drought seriously restricts agricultural production and reduces product yield and quality. At the same time, it can cause damage to the morphological development and physiological metabolism of the plant by preventing photosynthesis, respiration and stomatal movements of the plant during the plant growth process (Yang et al., 2021).

When the results obtained from the study were examined, no statistically significant difference was found in terms of survival rate under drought stress conditions (Table 2). The highest survival rate occurred in T1 (95.83%) and T2 (95.83%), and the lowest survival rate occurred in T3 (91.67%) applications. *Aronia melanocarpa* [Michx.] Elliot is not a very drought resistant species (Celik et al., 2022). The results obtained from our study support this opinion.

When the results obtained from the study under drought stress conditions were examined, the rooting rate was found to be statistically significant (Table 2; Figure 1). The highest rooting rate occurred in T1 (95.83%) and the lowest rooting rate occurred in T2 (37.50%) application. There were decreases in the rooting rate of plantlets in PEG applied nutrient media compared to the control. There are studies that decreased rooting rates with PEG applications. In a study conducted *in vitro*, when the responses to PEG induced drought stress in three different eggplant varieties were examined, it was stated that PEG applications reduced the rooting rate compared to the control (Zayova et al., 2017). In another study conducted under *in vitro* conditions, mannitol-induced drought stress conditions in five different potato cultivars significantly reduced the rooting rate compared to the control with applied mannitol concentrations, supporting our study (Dobránszki et al., 2003).

Table 2. Effect of PEG-8000 induced drought stress on morphological parameters.

Treatments	Morphological parameters			
	Survival Rate (%)	Rooting Rate (%)	Number of Root (per/plantlet)	Root Lenght (cm)
T1	95.83±7.22	95.83±7.22 a	24.08±1.81 a	5.29±0.49 a
T2	95.83±7.22	37.50±0.00 b	5.83±1.04 b	1.38±0.38 b
T3	91.67±7.22	50.00±12.50 b	4.50±1.00 b	1.25±0.15 b

*There is a 5% difference between means expressed with different letters in the same column (LSD)

When examined in terms of root number, a statistically significant difference was found between the applications. It was determined that the highest root number occurred in T1 (24.08 pieces/plantlet), and the lowest root number occurred in T3 (4.50 pieces/plantlet). With the decrease in the number of roots, the plant cannot get the substances it needs due to the decrease in the absorption of nutrients and minerals from the environment (Kocaçalışkan, 2003). In this study, the highest root number occurred in the control group and the decrease in the number of roots was observed with the increase in the severity of drought stress caused by PEG-8000, supporting this opinion. In some studies conducted under *in vitro* conditions, it was reported that the severity of drought stress increased with the increase in PEG concentration and subsequently a decrease in root development occurred (Mengesha et al., 2016; Gecene, 2020).

A statistically significant difference was found between the applications in terms of root length. The longest rooted plantlets occurred in the T1 (5.29 cm) application, and the shortest rooted plantlets occurred in the T3 (1.25 cm) application (Table 2; Figure 1). In studies conducted under drought stress conditions, the decrease in root length with increasing PEG concentrations is similar to the results obtained in our study. In studies conducted by some researchers, it was determined that root length decreased with increasing PEG concentration (Albiski et al., 2012; Meşe and Tangolar, 2019; Martínez-Santos et al., 2021). The obtained results support our study.

When the shoot length parameter was examined, a statistical difference was found between the applications. The longest shoots occurred in the T1 (4.28 cm) application, and the shortest shoots occurred in the T3 (0.79 cm) application. The main effect on the plant under drought stress conditions is the reduction of plant size (Salehi-Lisar et al., 2016). Optimum water is needed for plant growth and development. Cell growth in the plant is affected more by water scarcity than by cell division. Therefore, plant growth is inhibited by water deficiency under drought stress conditions (Sivritepe et al., 2008; Seleiman et al., 2019; Seleiman et al., 2021). Studies also support the results obtained from our study that shoot length decreases with increasing PEG concentration under drought stress

conditions. In a study conducted under *in vitro* conditions, five different PEG induced drought stresses were applied to different chickpea varieties, and it was reported that high concentrations of PEG negatively affected shoot length in chickpea varieties (Salma et al., 2016). In another study, four different PEG concentrations were applied to some wine grape varieties *in vitro* to determine their tolerance to PEG induced drought stress. The decrease in shoot lengths of all varieties with increasing PEG concentrations supports our study (Altıncı and Cangi, 2019).

A statistically significant difference was found between the applications in terms of leaf number. It was determined that the highest number of leaves occurred in T1 (24.67 pieces/plantlet), and the lowest number of leaves occurred in T3 (6.11 pieces/plantlet). In drought stress conditions, situations such as wilting of leaves, decrease in the number of leaves and change in leaf areas occur due to water deficiency (Yang et al., 2021). Plants tend to minimize water loss through transpiration by reducing the number of leaves depending on the severity and duration of drought (Jones and Cortlett, 1992). Similar results were obtained in the studies conducted. In a study conducted *in vitro*, five different PEG concentrations were applied to determine the tolerance of four grapevine rootstocks to drought stress. It was reported that as the PEG concentration increased, the number of leaves decreased (Mohsen et al., 2020). In another study, where morphological changes were examined against drought stress using five different almond varieties, the decrease in the number of leaves in all varieties occurred in parallel with the increase in drought stress, which supports our study (Zokaee-Khosroshahi et al., 2014).

Table 3. Effect of PEG-8000 induced drought stress on morphological parameters.

Treatments	Morphological parameters			
	Shoot Lenght (cm)	Number of Leaves (per/plantlet)	Plant Fresh Weight (g)	Plant Dry Weight (g)
T1	4.28±0.41 a	24.67±2.91 a	1.32±0.23 a	0.11±0.03
T2	1.28±0.19 b	10.89±0.69 b	0.45±0.09 b	0.09±0.01
T3	0.79±0.20 b	6.11±0.19 c	0.59±0.12 b	0.11±0.02

*There is a 5% difference between means expressed with different letters in the same column (LSD)

When the applications were examined in terms of plant fresh weight, a statistically significant difference was found between the applications. It was determined that the highest plant fresh weight occurred in the T1 (1.32 g) application, and the lowest fresh weight occurred in the T2 (0.45 g) application. Drought stress caused a serious decrease in the fresh weight of the plantlets. In order to prevent drying under drought stress conditions, stomata reduce their opening degree, and subsequently photosynthesis is affected by internal water deficiency. The amount of photosynthesis decreases with the decrease in the amount of carbon dioxide at the chloroplast level. The decrease in photosynthesis in plants under drought stress conditions results in the plants not storing sufficient amounts of dry matter in their bodies (Shangguan et al. 2000; Long et al. 2006; Yan et al. 2006; Lu et al., 2015). Results supporting this opinion were obtained in the studies. In a study, when the responses of five types of sunflower to drought stress were examined, it was reported that all varieties experienced significant decreases in plant fresh weight (Manivannan et al., 2007). In a study conducted to determine the drought tolerance of 5 kiwifruit species under *in vitro* conditions, the decreases in the fresh weight of plantlets under PEG induced drought stress conditions are parallel to our study (Zhong et al., 2018). When the applications were examined, no statistically significant difference was found between the applications in terms of plant dry weight. The highest plant dry weight was determined in T1 and T3 (0.11 g), and the lowest plant dry weight was determined in T2 (0.09 g).

Table 4. Effect of PEG-8000 induced drought stress on physiological parameters.

Treatments	Physiological Parameters		
	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Total Chlorophyll (mg/l)
T1	2.24±0.46 a	0.79±0.16 a	3.03±0.61 a
T2	1.26±0.44 b	0.42±0.15 b	1.68±0.59 b
T3	0.60±0.18 b	0.19±0.06 b	0.78±0.24 b

*There is a 5% difference between means expressed with different letters in the same column (LSD)

When the applications were examined in terms of chlorophyll a, a statistically significant difference was found between the applications. It was determined that the highest chlorophyll a content occurred in T1 (2.24 mg/l), and the lowest chlorophyll a content occurred in T3 (0.60 mg/l). As a result of the applications, a statistically significant difference was found between the applications in terms of chlorophyll b. The highest chlorophyll b content was determined in T1 (0.79 mg/l), and the lowest chlorophyll b content was determined in T3 (0.19 mg/l). A statistically significant difference was found between the applications in terms of total chlorophyll amount. It was determined

that the highest total chlorophyll content was obtained from T1 (3.03 mg/l), and the lowest total chlorophyll content was obtained from T3 (0.78 mg/l). As the PEG concentration increased, significant decreases occurred in chlorophyll a, chlorophyll b and total chlorophyll content. Chlorophyll is the basic component of the chloroplast in providing photosynthesis. Chlorophyll content is in positive interaction with photosynthesis rate. The possibility of high photosynthesis depends on high chlorophyll content (Anjum et al., 2011; Nurcahyani et al., 2019). Under drought stress, stomata close, resulting in a decrease in photosynthesis rate. Since insufficient photosynthesis is performed, decreases in chlorophyll content occur (Mahajan and Tuteja, 2005). Under drought stress conditions, chlorophyll content decreases and changes occur in chlorophyll a and b contents. Studies have also yielded results that support our study (Hancı and Cebeci, 2014; Kabay and Şensoy, 2016).

HIERARCHICAL CLUSTERING ANALYSIS (HCA)

HCA is a clustering method that provides the organization of groups and samples between groups. The HCA result is presented with a tree-shaped drawing showing the organization of samples and the relationships of samples (Lee and Yang, 2009; Granato et al., 2018). HCA was performed using 11 different parameters in line with the applications performed in the study (Figure 2). Applications were clustered as I and II. Parameters were grouped in 5 different ways as A, B, C, D and E. T2 and T3 applications were in cluster I, T1 application was in cluster II. T1 application was composed of 0% PEG concentration. In T1 application, root length, root number, shoot length, plant fresh weight, rooting rate, total chlorophyll, chlorophyll a, chlorophyll b and leaf number parameters were positively affected. T2 and T3 applications were composed of 1% and 2% PEG concentration. PEG concentrations in T2 and T3 applications negatively affected morphological and physiological parameters. Plant dry weight in group A; survival rate in group B; leaf number, chlorophyll a and b in group C; rooting rate and plant fresh weight in group D; shoot length, root number and root length parameters in group E. In the color scale given on the right, red represents the highest value, yellow and orange colors represent intermediate values, and blue represents the lowest values. Scale values vary between approximately -1.0 and 1.0. It was observed that the parameters in cluster II have high values (especially in T1 application), while parameters in cluster I have medium and low values in T2 and T3 applications. The highest value (red) is in T1 application (%0 PEG) in cluster II. In cluster I, plant dry weight in T2 application (%1 PEG) and survival rate in T3 application (%2 PEG) have the lowest value (blue) (Figure 2).

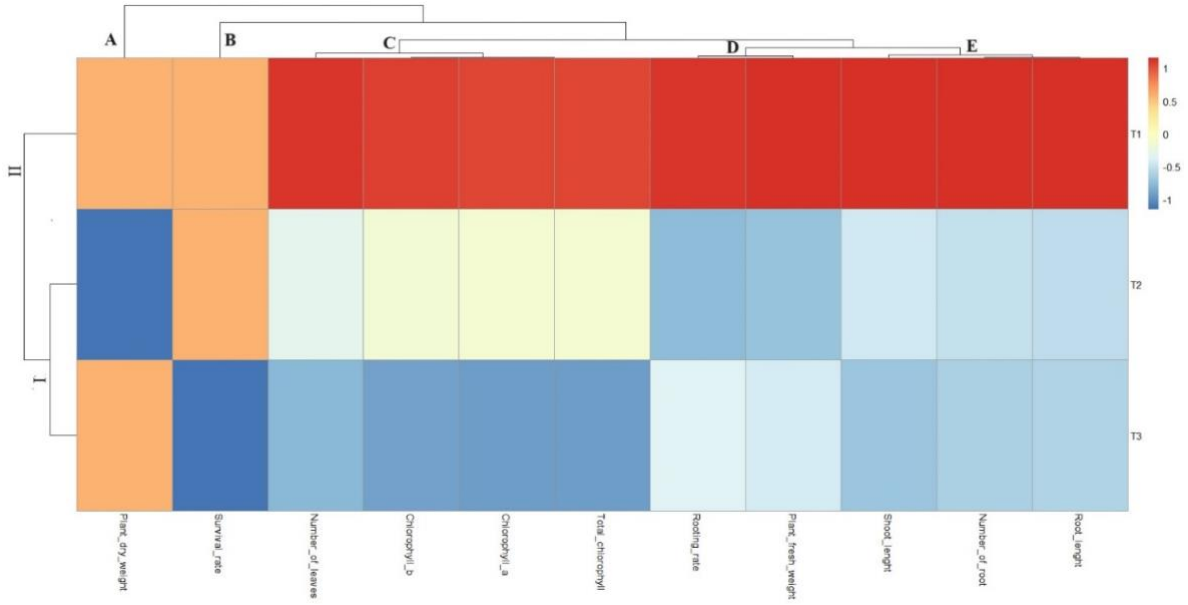

Figure 2. Heatmap for morphological and physiological parameters of plantlets exposed to PEG-8000 induced drought stress *in vitro*. T1: 0% PEG-8000, T2: 1% PEG-8000, T3: 2% PEG-8000. A, B, C, D and E represent the morphological and physiological parameters examined. I and II represent the applied PEG-8000 concentrations.

CONCLUSION

This study demonstrated that drought stress induced by PEG-8000 has significant effects on the growth and physiological parameters of *Aronia melanocarpa* 'Nero' under *in vitro* conditions. The findings indicate that increasing concentrations of PEG (1% and 2%) led to a decline in survival rate, rooting percentage, shoot and root length, leaf number, and chlorophyll content. These results highlight the sensitivity of *Aronia melanocarpa* 'Nero' to drought conditions, which limits its growth potential under water deficit.

In practical terms, these findings suggest that while *Aronia melanocarpa* is a valuable species due to its health benefits and antioxidant properties, its cultivation in areas prone to drought may require additional measures. The

use of irrigation systems or soil amendments to retain moisture could mitigate the negative effects of drought stress. Furthermore, selecting or breeding drought-tolerant cultivars of Aronia could be a promising strategy to enhance its resilience to water-limited environments.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that there is no actual, potential or perceived conflict of interest in this research article.

Author contribution

The contribution of the authors to the present study is equal. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

Ethics committee approval

Ethics committee approval is not required.

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Developing and modeling precipitation duration curves and determining spatial and temporal distributions of precipitation over different percentages of time

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Abstract
Determining the temporal distribution of precipitation is of critical importance for engineering hydrology, agricultural yield forecasting, and drought monitoring. The aims of the research carried out in this context were threefold: a) to develop *"precipitation duration curves"*, i.e., PDCs, of long-term total precipitation series, b) to investigate likely mathematical models of PDCs of each meteorological station studied, and c) to determine spatial and temporal distributions of precipitation that occur equalled or exceeded at 50% and 80% of the time. In line with the objectives, the PDCs were developed for each meteorological station. To this end, long-term annual precipitation data series were obtained from a total of 11 meteorological stations located in and around the borders of Sanliurfa province. PDCs of meteorological stations were modelled using the fifth-order regression equation at the 5% significance level. The mathematical forms of the developed model equations were used to predict precipitation amounts for each station at 50% and 80% of the time. The predicted precipitation data were mapped to delineate the spatial distribution of precipitation, and then hypsometric curves were generated from these maps. It was found that the standard errors (SE) of the *"precipitation duration curves"* models showed an increasing tendency as the standard deviation of the rainfall series increased. Regression analysis results showed that the SE values of the models change in direct proportion to the increase in extreme precipitation values. Considering the amount of precipitation that exceeds or equals 50 per cent of the time, it can be concluded that *"Semiarid"* climate characteristics prevail in the south of Hilvan meteorological station and *"Humid"* climate characteristics in the north. The precipitation, which occurs 80% time equaled or exceeded, indicates *"Arid"* climate characteristics in the southern parts of the Harran district and *"Semiarid"* climate characteristics in the northern parts of the study area. Considering the area averaged precipitation values corresponding to the two exceeded or equalled the percentage of time ratios, i.e., 50% and 80%, it can be concluded that *"Semiarid"* climate characteristics are dominant in Sanliurfa province and its surrounding geography.

Keywords: Regression analysis, Distribution of precipitation over time, Precipitation duration curve modelling, Drought, Sanliurfa

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INTRODUCTION

Water is crucial for life and plays an important role in biological, geological and chemical processes. The total amount of water in the world is about 1.338 billion km^3 (Dikmenli et al., 2024). Although 97.5% of this amount is found as salty water in the oceans and seas and the remaining 2.5% as fresh water in solid, liquid and gaseous form, only 0.4% of the freshwater available as surface water (lakes, rivers and swamps) is usable. Furthermore, only 0.001% (12.900 km^3) out of 1.338 billion km^3 of water on Earth is present as water vapour in the atmosphere (Gleick, 1996; USGS, 2024; NASA, 2024a). Of the 577000 km^3 of water in the hydrological cycle, 458000 km^3 falls to the ocean and 119000 km^3 to the land as precipitation. The amounts of evaporation from the ocean surface

and land are 505000 km^3 and 72000 km^3, respectively (Shiklomanov, 2009). As it can be understood, the amount of fresh water available in our world is very limited, and the spatiotemporal distribution of precipitation as well as freshwater resources is irregular. Just like everywhere else, the distribution of precipitation in Turkiye is remarkably irregular. Topographical differences and the resulting 7 different climate zones are the main reasons for this irregularity in precipitation distribution.

Studies carried out in recent years have shown that Turkiye's long-term average annual rainfall has been 574 mm. Although the eastern Black Sea region receives the most precipitation (1200-2500 mm/year), the central Anatolian region (around Lake Tuz) receives the least (250-300 mm/year) (Cetin, 2020). Total annual rainfall yields 501 billion cubic meters of water in Turkiye each year. Of this water, 274 billion m^3 is returned to the atmosphere by evapotranspiration, 69 billion m^3 of this water recharges groundwater system, and 158 billion m^3 is discharged into the seas and lakes by river or surface runoff (Çiçek and Ataol, 2009; Usta, 2016). Turkiye has a technical and economic water potential of 112 billion m^3 including 98 billion m^3 of surface water and 14 billion m^3 of groundwater. However, 57 billion m^3 of the 112 billion m^3 water potential can be utilised. Of this amount, 44 billion m³ is used in irrigation (Cetin, 2020) and 13 billion m³ is used for domestic and industrial purposes (DSİ, 2024). According to the Falkenmark water stress index, Turkey is among the “water scarce” countries with an annual water potential of 1000-1500 m^3/year per capita (Wolf et al., 2003; Aydın et al., 2017). Recent studies have shown that 40% of the world's population will be living in regions with severe water shortages by 2035, which will cause the ecosystems that provide freshwater resources to be increasingly compromised (Guppy et al., 2017). Under these conditions, considering the undeniable importance of precipitations in water management in Turkey, determining the distribution of precipitation over time is a much-needed issue. The irregular distribution of precipitation in time and space in Turkiye, the determination of the temporal distribution of precipitation series, observed at a meteorological station, using the methodology of the development of *"flow duration curves"* can have many practical advantages.

Turkey's location between temperate and subtropical regions, together with the influence of the Mediterranean macroclimatic zone (Türkeş, 2021), causes it to be more exposed to extreme weather events in summer and winter (Yılmaz et al., 2021). The average temperature in Turkey is expected to increase by 2.65 °C in the next 100 years due to changes in extreme weather events such as drought and heat waves caused by global warming. Moreover, spatial and temporal temperature distributions already pose risks (Tonkaz and Cetin 2007; Aksu, 2021; Coşkun et al., 2021; CCP, 2024; Çeliktopuz, 2024). Climate change is not only about increasing temperatures and droughts but also about the spatial and temporal distribution of precipitation across the globe. Especially, causing extreme precipitation events to occur more frequently in different parts of the world (Nordling et al., 2024; NASA, 2024b). Similarly, the number of recurring extreme wet and dry events in Turkey is increasing day by day (Sılaydın Aydın and Kahraman, 2022; Keskiner and Şimşek, 2024). According to climate projections, precipitation is expected to decrease in all river basins of Turkey, and the *Euphrates-Tigris* basin in the Southeastern Anatolia region is among the basins that will be most affected by climate change. Therefore, Turkey is one of the countries that will suffer the most from the negative impacts of climate change (MGM, 2014; Demircam et al., 2017) and it would be overly optimistic to expect that long dry periods will not be experienced in drought-prone regions in the future. Moreover, the fact that the Southeastern Anatolia Project (GAP), one of Turkey's most important development projects with an irrigable agricultural area of 1.78 Mha, is located in a semi-arid region makes it inevitable that the agricultural sector will be negatively affected by drought events (Küçüközcü and Avcı, 2020; Keskiner and Cetin, 2023a; Kılınçoğlu et al., 2021).The gradual impact of drought on many economic sectors and the unpredictability of its onset and end - the inherent uncertainty of a drought event - have a multiplier effect on drought-related losses. Many drought indices have been developed to monitor and mitigate the negative effects of drought and the most important variable used in the calculation of these indices is precipitation (Erinç, 1965; Aydeniz, 1985; McKee et al., 1993). Therefore, determining the spatial and temporal distribution of precipitation is of vital importance for risk management in agricultural production. In rain-fed areas, such as those around the province of Sanliurfa in the GAP project area, rainfall is the only form of insurance for agricultural production. In this context, 11% of Turkiye's economically irrigable area and about 50% of the GAP project irrigation areas are within the borders of Sanliurfa province (Keskiner and Cetin, 2023b), which is exposed to drought risk.

The main objectives of this study were to: a) develop *"precipitation duration curves"*, i.e., PDCs, of long-term total precipitation series, b) to investigate likely mathematical models of PDCs of each meteorological station studied, c) determine spatial and temporal distributions of precipitation that occur equalled or exceeded at 50% and 80% of the time, d) derive *“precipitation hypsometric curves”* for the specified time ratios to figure out the changes in the areal average values of precipitation within the province.

MATERIALS AND METHODS

Description of Study Area and Data Source

This study was conducted in and around Sanlıurfa province, which is located between 37˚49' 12"- 40˚10' 00" E longitude and 36˚41' 28" - 37˚57' 50" N latitude in the Southeastern Anatolia Region of Turkiye. The study area covers an area of approximately 19 242 km² (HGM, 2022). Topographically, the south of Sanliurfa is a plain. In

the north, Karacadag Mountain (1957 m), which stretches across Diyarbakır and Sanliurfa provinces, is located in the Siverek district. Mandal Hill (1895 m) is the highest point in Sanliurfa. Altitude decreases from Siverek towards the Syrian border, it is about 360 meters around Akcakale and Ceylanpınar. The low elevation along the Syrian border in Sanliurfa and in the inland areas from the border to the north causes extremely hot air masses originating from the Basra Low-Pressure Centre to be effective in the region during the summer period. This situation aggravates the occurrence of droughts that stretch from south to north in Sanliurfa. In the province of Sanliurfa, which is dominated by a continental climate on a macro scale, the long-term average temperature is around 18.6 ^{0}C. The long-term average of the total annual precipitation varies from 558 mm in Siverek, to 448 mm in Sanliurfa, to 285 mm and 295 mm in the districts of Akcakale and Ceylanpınar, respectively (İrcan and Duman, 2021; Keskiner and Cetin, 2023a). Understandably, annual precipitation amounts decrease significantly from north to south in the study area. Long-term annual total precipitation series acquired from meteorological stations of Ergani, Diyarbakır, Adıyaman, Siverek, Bozova, Mardin, Sanliurfa, Gaziantep, Birecik, Ceylanpınar and Akcakale, located in and around Sanliurfa province (Figure 1) and operated by the General Directorate of Meteorology, were used in this study. The location of the meteorological observation stations in the projected coordinate system was calculated in accordance with the D_WGS_1984_UTM_Zone_37N reference surface. In Table 1, the long-term averages of total annual precipitation values and some attribute information of meteorological observation stations are given in latitude order.

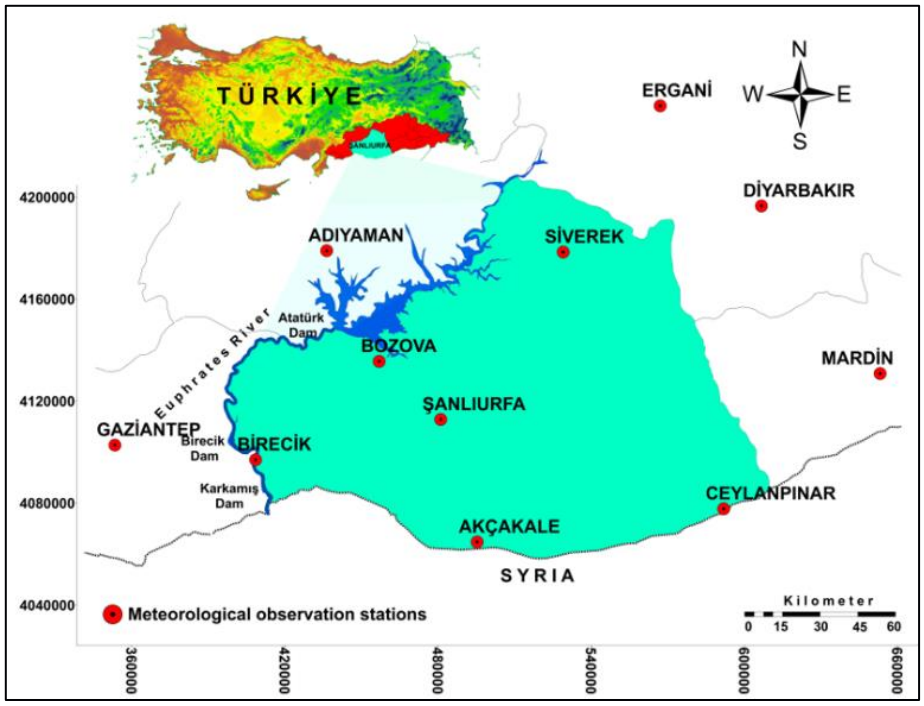

Figure 1. Location of the study area in Turkiye and spatial distribution of meteorological observation stations over the study area

Table 1. Some attributes of the meteorological stations used in the study

Station Name	Latitude (^{0})	Longitude (^{0})	Altitude (m)	Observation Period	Average Annual Precipitation (mm)
Ergani	38.267	39.766	986	1965-2023	735
Diyarbakır	37.909	40.213	680	1950-2023	494
Adıyaman	37.755	38.277	672	1963-2023	709
Siverek	37.752	39.329	801	1966-2023	558
Bozova	37.365	38.513	622	1982-2023	387
Mardin	37.310	40.728	1040	1955-2023	644
Sanliurfa	37.160	38.786	550	1955-2023	448
Gaziantep	37.058	37.351	854	1959-2023	557
Birecik	37.013	37.971	347	1966-2023	354
Ceylanpınar	36.841	39.933	360	1957-2023	295
Akcakale	36.727	38.947	365	1965-2023	285

Developing Precipitation Duration Curves

In this study, the method used in obtaining *flow duration curves* (Beşiktaş, 2010; Demir and Tona, 2021) was employed to develop the *precipitation duration curves* (PDCs). A PDC is a cumulative frequency curve showing the percentage of time when precipitation is greater than or equal to a certain P_i value. In other words, PDC is a relationship between precipitation value and the exceeded or equalled percentage of the time. By using these curves, the precipitation exceeding a certain percentage of the time can be determined practically or the percent of time indicated precipitation was equaled or exceeded may be estimated for hydrological design purposes. To this end, the *precipitation duration curve* may be derived by labelling the vertical axis with precipitation values *(P)* and the horizontal axis with the percentages of time when the precipitation considered is equal to or greater than a certain value. The choice of time unit is determined by the purpose for which the curve is to be used (Bayazıt, 1999; Karakoyun et al., 2018). In this study, the *precipitation duration curve* for each meteorological station was derived by imitating the *flow duration curve* development procedure, which is widely used in hydrology. In order to obtain the *precipitation duration curve*: a) Precipitation time-series of a meteorological station is ordered from largest to smallest, b) The number of rows (m_i) of each P_i precipitation is obtained, c) The rank number of precipitation (m_i) is divided by the total number of observations *(N)* to calculate the exceedance percentage or exceedance rate (AO_i) of the considered precipitation P_i (Equation 1), d) The *precipitation duration curve* of the meteorological station in question is obtained by plotting the P_i values on the vertical axis and the AO_i ratios on the horizontal axis (Raudkivi, 1979).

$$AO_i = \frac{m_i}{N} * 100 \quad (1)$$

Regression Analysis

Precipitation duration curves (PDCs) can be described in mathematical terms by means of a polynomial. In this study; the regression model shown in Equation 2 was used for modelling the *PDCs*. For model selection, the *"CurveExpert"* software (Hyams, 2020) was used due to its ease of use. Mathematical forms of *PDCs* have been obtained with linear regression models up to the fifth order. The statistical significance of the parameters in Equation 2 and the model was determined by analysis of variance (Ryan and Cryer, 2005) at α=0.05 level of significance and the appropriate model was selected based on the highest R^2 and the *smallest model standard error (SE)*.

$$P_i = b_0 + b_1\, AO_i + b_2\, AO_i^{\,2} + b_3\, AO_i^{\,3} + b_4\, AO_i^{\,4} + b_5\, AO_i^{\,5} + e_i \quad (2)$$

Where: P_i is the dependent variable (annual total precipitation, mm); AO_i is the independent variable (the percentage of time in the long-term total precipitation series that indicates that the precipitation was equal to or higher during the observation period); b_i are the regression coefficients and e_i are the error term.

Development of "*Precipitation Hypsometric Curves*"

Hypsometric curves have been widely used to simulate the spatial distribution of regional variables such as surface runoff (Vivoni et al., 2008), drought indices (Keskiner et al., 2020), salinity (Cetin, 2003), groundwater (Cetin and Kirda, 2003; Keskiner et al., 2018), etc. In order to develop the *precipitation hypsometric curves*, first, the precipitation value P_i at the specified time percentage (x%) is estimated by using the mathematical model of the *PDC* of each meteorological station. A map is then drawn up using the estimated $P_{i,x\%}$ values as the basis. Eventually, the *precipitation hypsometric curve* for $P_{i,x\%}$ is derived by using the value of the precipitation at each pixel in the map and the areas that are covered by this value. In this study, the precipitation values exceeded 50% and 80% of the time ($P_{i,50\%}$: exceeded precipitation value of station *i* at 50% of the time, mm) were estimated by using the *precipitation duration curve* model of each station. These estimated precipitations were mapped at 200 m x 200 m resolution by inverse distance weighted interpolation technique (Liu et al., 2021; Keskiner and Cetin, 2023a) in a geographic information systems environment and their areal distributions were obtained. With the help of these maps, precipitation hypsometric curves were developed for 50% and 80% time ratios by applying the method (Chow et al., 1988) to obtain the catchment height-area relationship.

RESULTS AND DISCUSSION

Descriptive Statistics of Precipitation Series

Some characteristic values of meteorological observation stations are given in Table 1. Descriptive statistics of the annual total precipitation series of meteorological stations considered were calculated and presented in Table 2. Given the latitude at which the stations are located, it is noteworthy that the averages ($\overline{P}$) of Ergani-Siverek-Sanliurfa-Birecik-Ceylanpınar-Akcakale stations decrease with latitude in precipitation values from north to south, although not with a constant slope due to the effect of factors such as topography, aspect, etc. (Table 2). In other words, average precipitation observed at the stations tends to decrease from north to south. When $\overline{P}$ and median values of the precipitation series were compared, the median values of all stations were found to be smaller than

the mean, indicating that the precipitation series are characterised by a right-skewed distribution. This conclusion was confirmed by the values of the coefficient of skewness *(C_{skew})*. Given the C_{skew} coefficient, Akcakale station was found to have the largest skewness coefficient (C_{skew}*=1.22*). The conclusion was that it would be more accurate to use the *Med* value of the data set as a representative value in project design studies (Hayes, 2007) instead of using $\bar{P}$ values in Akcakale station. It was found that the magnitude of the C_{skew} coefficient and the *STD* value were not compatible. However, it is clear that the magnitude of the range *(R)* of the data set directly affects the *STD* value. It was determined that the *STD* value of Ergani station *(STD=195.4)*, which has the largest *R*-value *(R=1006.1 mm)*, is represented by the largest value after Mardin station *(STD=217.5*). When all the stations used in the study are taken into consideration, it can be said that the similarity of precipitation between the years is low in Ergani, Mardin and Adıyaman stations since the difference between the average precipitation values and the observed precipitation values is the largest. When minimum precipitation values (P_{min}) are considered; Ceylanpinari (P_{min})=70.6 mm) and Akcakale stations (P_{min})=108.4 mm) are predicted to be more at risk of drought than other stations (Keskiner and Cetin, 2023a). Considering the maximum precipitation (P_{max}) values of the stations, it was concluded that extreme precipitation was effective (Kılınç et al., 2023) in Ergani (P_{max}=1351.9 mm), Mardin (P_{max}=1193.9 mm) and Adıyaman (P_{max}=1152.8 mm) stations. When the coefficient of variation *(CV)* is analysed, the variability in precipitation series at all stations was found to be *CV≥25%*. This statistic provided evidence of significant variability in the temporal distribution of precipitation. When the *STD* values and averages of the precipitation series are evaluated jointly, it is understood that the variability varies between 25%-37%. Therefore, the variability in annual precipitation observed in and around Ceylanpınar and Akcakale stations is larger compared to other stations. As explained by Nordling et al. (2024), the reason of this situation is because precipitation diminishes the closer you get towards the Syrian border, the standard deviation values do not decrease at the same rate and the high variation observed in the precipitation series. Conversely, the high CV at these stations may reflect the high drought impact. The higher the *CV*, the greater the dispersion in the precipitation events, indicating that data points in the precipitation series are more dissimilar and inconsistent, and that extreme values become more likely.

Table 2. Descriptive statistics of data sets of yearly precipitation totals observed in meteorological stations

Station Names	$\bar{P}$ (mm)	*Med* (mm)	*STD* (mm)	P_{min} (mm)	P_{max} (mm)	*R* (mm)	C_{skew}	C_{kurt}	*CV* (%)
Ergani	734.8	721.8	195.4	345.8	1351.9	1006.1	0.68	0.95	27
Diyarbakır	493.7	476.1	136.0	146.3	854.7	708.4	0.14	0.14	28
Adıyaman	708.9	678.0	192.8	364.8	1152.8	788.0	0.42	-0.59	27
Siverek	557.6	537.3	155.5	255.0	893.0	638.0	0.35	-0.56	28
Bozova	386.5	354.8	109.9	177.2	655.9	478.7	0.75	0.18	28
Mardin	644.3	627.8	217.5	247.6	1193.9	946.3	0.52	-0.24	34
Sanliurfa	448.0	425.3	147.0	160.5	854.7	694.2	0.80	0.59	33
Gaziantep	557.1	540.4	140.7	325.1	994.0	668.9	0.68	0.55	25
Birecik	354.2	337.0	102.8	187.6	614.0	426.4	0.64	-0.02	29
Ceylanpınar	294.6	273.7	107.9	70.6	546.1	475.5	0.46	-0.31	37
Akcakale	284.9	269.9	105.8	108.4	646.2	537.8	1.22	2.11	37

Modelling of Precipitation Distribution Over Time

The first step in developing *precipitation duration curves (PDC)* for each station used in the study was to plot the annual precipitation value of a station versus *the exceeded or equaled percentage of time* over a given period. A 5th-order polynomial (Equation 2) represents the mathematical forms of the resulting *PDC* distributions. Figure 2 shows the 5th-order regression models for the precipitation duration curves and the scatterplot fits of the P_i-AO_i values. Table 3 shows the model coefficients, correlation coefficients *(r)*, coefficients of determination *(R^2)* and standard errors *(SE)* of the regression models fitted to the *PDC*s. One of the most remarkable characteristics of the models is that the model *SE* values of Mardin (Pmax=1193.9 mm), Ergani (Pmax=1351.9 mm), Gaziantep (Pmax=994.0 mm) and Adıyaman (Pmax=1152.8 mm) stations increase in parallel with the size of maximum precipitation observations in the time series. In other words, the larger the maximum precipitation in te series, the larger the *SE* value of the *PDC* model. In turn, the SE values of these stations are 19.72 mm, 19.362 mm, 16.132 mm and 13.27 mm, which are larger than the model standard error of the other stations, respectively. This may be due to the fact that the maximum precipitation data in the precipitation series may be an outlier. However, it can be said that the maximum precipitation is effective in increasing the error of the regression model. Therefore, our recommendation is that before analyses are performed, outliers must be removed from the data. It was found that the precipitation series of Bozova, Birecik, Ceylanpınar and Akcakale stations, where the SE values of the *PDC* regression models of the stations are less than 10 mm, have the smallest STD values. The SE values of the *PDC* regression models of the precipitation series of these stations are 9.91 mm, 6.04 mm, 9.00 mm and 9.34 mm,

respectively. The Siverek station, which is located in a topography with a high difference in altitude compared to the other stations, does not show any similarity to this structure with a value of STD=155.5 mm, although SE is 8.36 mm. However, as pointed out by Carter (2013), the small standard deviation (STD) values of the precipitation series are an indicator of low variability in the data, which can be said to contribute to reducing the model error. In other words, the variability of the data (STD and CV) affects the prediction error (SE) of the models.

Table 3. Regression model coefficients and model goodness-of-fit statistics representing the precipitation duration curves of the observation stations

İstasyonlar	b_0	b_1	b_2	b_3
Ergani	1381.725562756870	-57.1761359002948	2.36553998142426	-0.0486887247871676
Diyarbakır	852.631827388411	-25.1970616562749	1.01487445375872	-0.0231161714340396
Adıyaman	1172.872452246460	-20.8384015641572	0.47465384293105	-0.0080878250930805
Siverek	919.250273509813	-14.6518299919079	0.29673242451598	-0.0056301326359867
Bozova	706.451732731845	-18.7093008025387	0.54448144215318	-0.0102563571054207
Mardin	1238.566182448110	-39.1954490683444	1.46302277930733	-0.0317335856990392
Sanliurfa	920.549972447308	-35.6600798800292	1.25726081486108	-0.0240060035833074
Gaziantep	979.758067858816	-31.7162156414739	1.19366620090969	-0.0239670327815119
Birecik	639.605983402985	-15.4835267305092	0.35034635537457	-0.0042324696024038
Ceylanpınar	570.724095653920	-14.5408265637939	0.42707168364310	-0.0089058185584778
Akcakale	681.932075897924	-36.5291137524379	1.50150095617332	-0.0311388803925949

İstasyonlar	b_4	b_5	r	R^2	*SE (mm)*
Ergani	0.000465714672729440	-0.000001684071610906910 0	0.995	0.991	19.36
Diyarbakır	0.000250145231017204	-0.000001020765632050570 0	0.997	0.994	10.49
Adıyaman	0.000075366969471655	-0.000000289928046224835 0	0.997	0.995	13.27
Siverek	0.000064712760415137	-0.000000299573400243561 0	0.998	0.997	8.36
Bozova	0.000102334755298633	-0.000000405652349854776 0	0.996	0.992	9.91
Mardin	0.000327241949987971	-0.000001269288755835610 0	0.996	0.992	19.72
Sanliurfa	0.000225087858060502	-0.000000825208893694045 0	0.997	0.993	12.49
Gaziantep	0.000227858174113607	-0.000000825610496322160 0	0.993	0.987	16.13
Birecik	0.000023528243097752	-0.000000052595871424122 7	0.998	0.996	6.04
Ceylanpınar	0.000096877886073248	-0.000000408111918207387 0	0.996	0.993	9.00
Akcakale	0.000304646044900108	-0.000001127330505952810 0	0.996	0.992	9.34

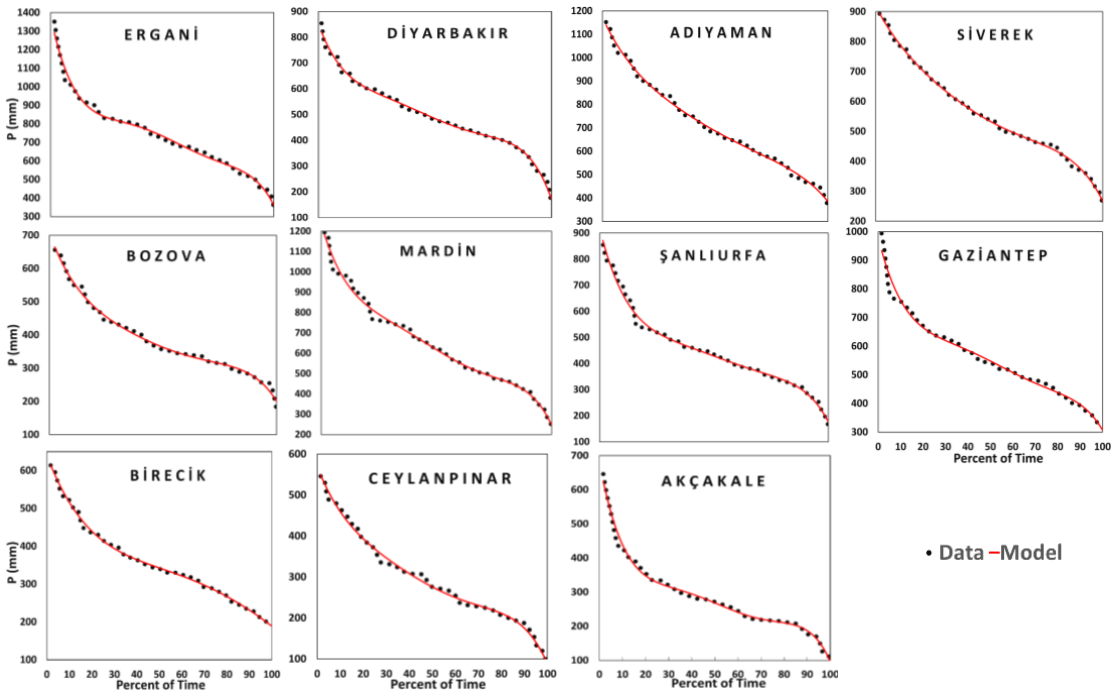

Figure 2. Annual precipitation values versus the exceeded or equalled percentage of time with regression model fits (*PDCs*) of the meteorological stations used

Spatial Distribution of *Precipitation Duration Curves*

Evaporation, which is very effective in the cause of drought, is highly dependent on temperature. However, drought indices such as *standardized precipitation index (SPI), percent of normal index (PNI)* etc. define drought severity by using only precipitation values. In the definition of climate, aridity also plays an important role in agricultural water management as well as rainfed farming. Therefore, there are studies by IPCC (2007), Huang et al. (2016) and Thomas (2011) in which aridity is grouped based on mean annual precipitation. For example, Holzapfel (2008), referring to areas with high evaporation during the growing season, classified drought into 4 groups based on *total annual mean precipitation* (Table 4). Indeed, Sanliurfa is one of the provinces with low relative humidity, high average temperature and high evaporation demand during the growing season (Elgalı, 2020; Ürün et al., 2023). In this context, the values of precipitation expected to be exceeded or equalled 50% and 80% of the time were calculated for Sanliurfa using the *PDC* models. Table 4 has been taken into account in the interpretation of the spatial and temporal distribution of these calculated precipitations. Figure 3 shows the precipitation that was exceeded (present) in 50% of the cases, depending on the observation period of the stations. Those curves given in Figure 3 may be used to estimate the percent of time that a specified precipitation will be equalled or exceeded in the future or to estimate discharge for a given percent of duration. Such estimates are required for drought mitigation studies.

Table 4. Grouping drought/climate categories in areas with high evaporation during the plant-growing season (Holzapfel, 2008)

Type of Aridity	Amount of mean annual precipitation (mm)
Extreme Arid	less than 60-100 mm
Arid	from 60-100 to 150-250 mm
Semiarid	from 150-250 to 250-500 mm
Nonarid (Mesic)	above 500 mm

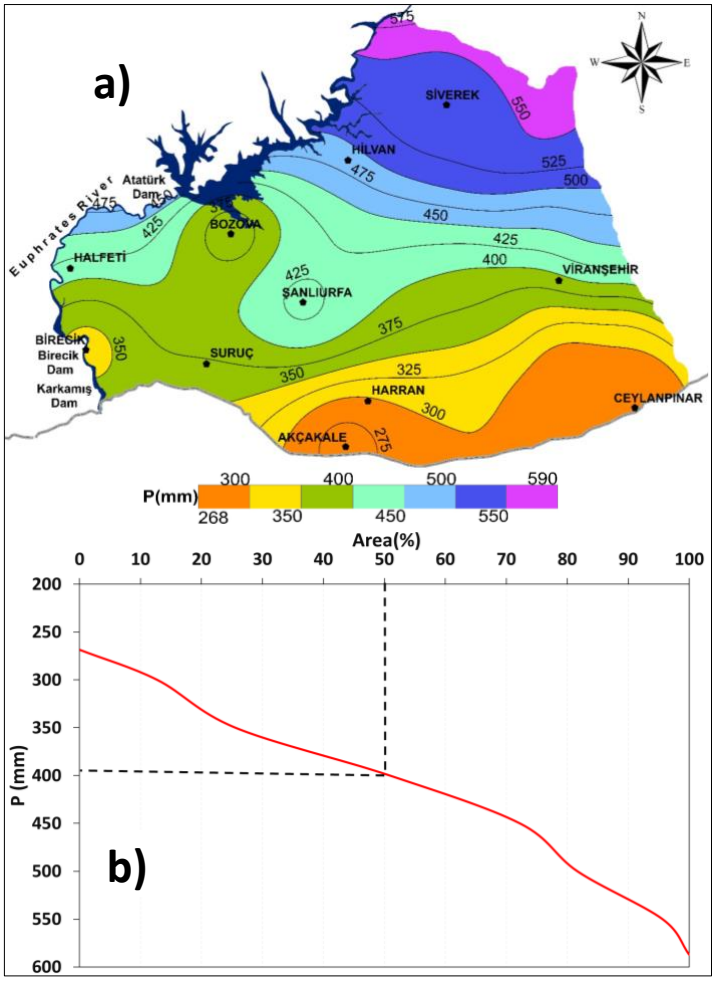

Figure 3. a) Spatial distribution of precipitation values exceeded 50% of the time $(P_{50\%})$ and b) *Precipitation hypsometric curve* for $P_{50\%}$

As can be seen in Figure 3, the precipitation values that occur 50% of the time in Sanliurfa can be considered as the boundary line (500 mm precipitation contour) dividing the north and south of Hilvan into two distinct climate types of *"Humid*" and *"Semiarid"*, respectively. From the south of Hilvan to the Syrian border, the *"Semiarid*" climate character is dominant, while the amount of precipitation decreases from north to south. This remarkable decrease in precipitation can be attributed to the high level of aridity prevailing in Syria. Taking into account the criteria given by Santos et al. (2022), which are based on precipitation that falls 50% of the time, it can be concluded that the expected yield of agricultural production cannot be achieved without irrigation in the south of Hilvan. On the other hand, given the remaing 50% of the time, the north of Hilvan, Siverek and its surroundings are under the influence of the humid climate. From the analysis of the hypsometric curves of precipitation, it can be concluded that the average areal precipitation in the province of Sanliurfa is 390 mm 50% of the time and that it is under the influence of a *"Semiarid"* climate regime (Keskiner and Cetin, 2023b).

Likewise, the spatial and temporal distribution of precipitation over 80% of the time is determined using the *PDC* models, as shown in Figure 4. Significant differences were found in the spatial distribution of the precipitation amounts that occurred 80% of the time compared to precipitation amounts that occurred 50% of the time. It was found that the amount of precipitation falling 80% of the time decreased and the severity of drought in the study area increased. Especially around the settlements of Harran, Akcakale and Ceylanpınar, exceeded precipitation of 250 mm or more in 80% of the time means that the precipitation falling on the site in the remaining 20% of the time will be less than 250 mm, indicating that the region can expect severe rainfall deficiencies once in five years (İrcan and Duman, 2021). This constitutes evidence that Arid climate characteristics prevail in the region 20% of the time. It was found that the Harran meteorological station and its surroundings constitute a border between *"Semiarid"* and *"Arid"* climate characteristics. In turn, findings are a precursor of the "*Arid*" climate type in the southern parts of the study area in the future. On the other hand, in the north of Harran district, it was determined that the humidity continued to increase towards Siverek and the *"Semiarid"* climate characteristics were effective in the north of Harran. However, considering the amount of precipitation exceeded 80% of the time, it is an undeniable fact that the expected yields cannot be obtained without irrigating agricultural crops. This means that dry farming will be even more at risk in the future. The average value of precipitation exceeded 80% of the time is 320 mm. For this reason, Sanliurfa and its surroundings have a *"Semiarid"* (Keskiner and Cetin, 2023a) climate regime 80% of the time, and an *"Arid"* climate type is preponderant for the remaining 20% of the time.

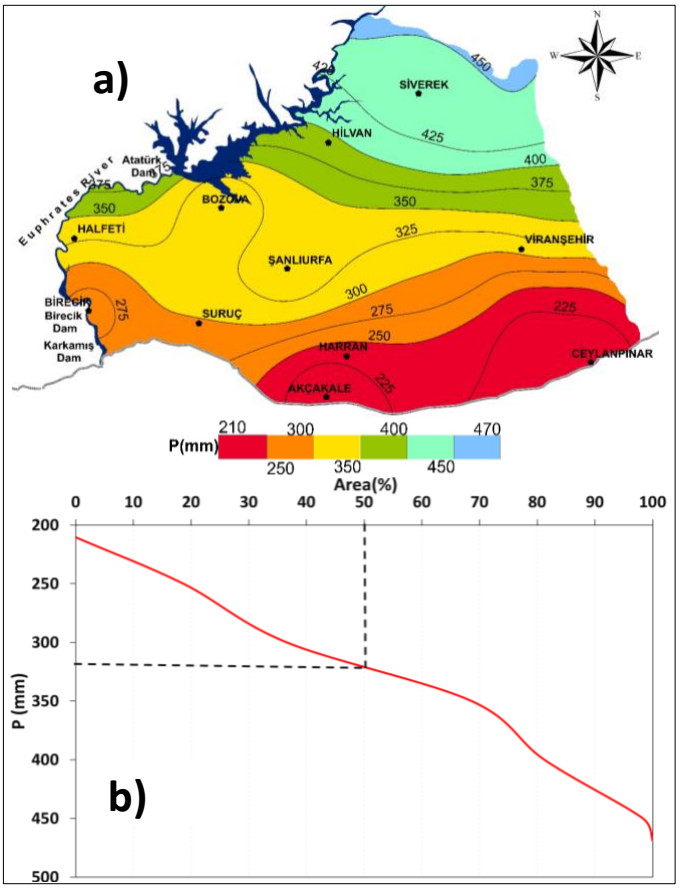

Figure 4. Spatial distribution of precipitation values exceeded 80% of the time $(P_{80\%})$ and b) *Precipitation hypsometric curve* for $P_{80\%}$

CONCLUSIONS AND RECOMMENDATIONS

The South East Anatolia Project (GAP) aims to irrigate an area of 1.78 million hectares. Approximately 940 000 ha of this irrigable area is within the borders of Sanliurfa province. Therefore, it is important to know the distribution of precipitation that is exceeded in a certain percentage of time in and around Sanliurfa province in terms of irrigation water management. Sanliurfa province is expected to be more affected by a potential drought than other provinces located in the GAP area due to its large irrigated areas. Within the framework of this study, the *precipitation duration curves* of the long-term total annual precipitation series of each meteorological observation station located in the GAP area have been developed and these curves have been mathematically modelled. Spatial and temporal distributions of the precipitation amounts exceeded 50% and 80% of the time were determined using the model equations obtained for each station. *Precipitation hypsometric curves* were derived from the maps drawn for the specified time ratios, i.e., the exceeded or equalled percentage of time. With the help of precipitation hypsometric curves, the spatial variation of areal means of total annual precipitation exceeded 50% and 80% of the time in the study area was revealed by generating precipitation maps. Conclusions that were drawn in light of the results of this study are listed below:

- ✓It was observed that as the STD values in the annual precipitation series increased, the SE values of the *precipitation duration curve models* also increased. The SE values of the models were directly proportional to the increase in maximum precipitation.
- ✓It is recommended that the outliers are analysed prior to performing the PDC analysis and that the outliers are removed from the data if sufficient evidence can be found.
- ✓Model equations for precipitation duration curves can be used to instantly calculate or estimate precipitation amounts that exceed a certain percentage of the time. In this context, it was determined that rainfall exceeding 50% of the time had a drought-reducing effect. On this time scale, the province of Sanliurfa is characterised by *"Semi-arid*" and *"Humid"* climate types which are harbingers of ongoing drought events.
- ✓A reduction in the amount of precipitation that occurs 80% of the time, increased the severity of drought in the study area. It was found that *"Arid"* climate characteristics were dominant in and around the Harran, Akcakale and Ceylanpınar meteorological stations due to the impacts of severe arid conditions in Syria's territory. Considering the precipitation in this time scale, "Semiarid" and "Arid" climate characteristics prevailed in the province and "Humid" climate characteristics were not observed.
- ✓Considering the areally averaged precipitation occurring in these two time scales; it is concluded that the *"Semiarid"* climate characteristic is dominant for Sanliurfa province.
- ✓*Precipitation duration curves* and *precipitation hypsometric curves* can be used operationally in and around the study area by practitioners in water resource planning, irrigation management and drought monitoring studies, etc.

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.

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Use of the radicle emergence test (RE) to estimate germination and emergence potential in sponge gourd (*Luffa aegyptiaca* Mill.) seed genotypes

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Abstract

The study was carried out to test whether the radicle emergence (RE) test could be used to rank 12 Luffa (*Luffa aegyptiaca* Mill.) seed genotypes according to total (TG, %) and normal (NG, %) germination percentages in laboratory conditions (25°C, 14 days, dark), and mean germination time (MGT, day), seedling emergence percentage (SE, %), seedling shoot weight (SSW, g/plant) and hypocotyl thickness (HT, mm/plant) in climatic room conditions (23±2°C, 70% relative humidity), in peat moss medium after 15 days. RE (2 mm radicle) counts were made at 24, 48, 72, 96, 120 and 144 hours at 25°C in the dark after germination was set up. RE values were correlated to seed germination and seedling quality parameters. The highest correlation values occurred after 72h and onwards. RE 72h and seed quality parameters were regressed and were found to be significantly related to TG (R^2=0.732, P<0.001), NG (R^2=0.751, P<0.001), MGT (R^2=0.842, P<0.001), SE (R^2=0.754, P<0.001), SSW (R^2=0.349, P<0.05), and HT (R^2=0.757, P<0.001). The potential of the RE test to discriminate between Luffa genotypes according to their germination and emergence potential is discussed.

Keywords: Luffa genotypes, RE test, Germination, Seedling emergence, Seed vigour

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INTRODUCTION

The sponge gourd (*Luffa*) belongs to the Cucurbitacea family and is cultivated widely as a vegetable in India, China and Asian countries. Its immature fruits are used as vegetables and the mature fruits as fibres. The genus *Luffa* consists of ten species but specifically *Luffa aegyptiaca* Mill. and *Luffa acutangula* Roxb. are economically cultivated in different parts of the world (Marr et al., 2005).

Vegetable transplant grafting has been widely practiced in Cucurbitaceae for various purposes, including the enhancement of tolerance to abiotic and biotic stresses (Savvas et al., 2010; Lee et al., 2010; Jang et al., 2022). Grafting is also considered to be an environment-friendly operation (Rivard and Louws, 2008) for sustainable vegetable growing systems. One of the main purposes of grafting is to improve tolerance to soil pathogens such as Fusarium wilt as well as to low temperature and high salinity (Yetisir et al., 2007; Mavi et al., 2006). In this way, rootstocks that are tolerant to such soil borne diseases can be valuable. Luffa species have the merit of nematode tolerance and have the potential to be used as a rootstock for watermelons and melons (Schwarz et al., 2010). For successful grafting, high and uniform seedling emergence and longer hypocotyl length are important since the occurrence of more similar sized seedlings gives a chance for the production of a higher number of grafts at the same time (Mavi et al., 2006). Thus, estimation of seed germination and emergence potential of seed lots/genotypes is valuable. This study highlights the importance of uniform seedling emergence and hypocotyl length for successful grafting. This is directly relevant to focus on optimizing seedling growth conditions. Estimating the seed germination and emergence potential of different genotypes is a critical aspect of my research, as it allows for better planning and execution of grafting procedures, ultimately leading to higher grafting success rates.

Ability to emerge successfully in a wide range of environments is reflected in the seed vigour characteristics of any lot (Powell, 2022). Vigorous seed lots produce higher seedling emergence percentages and stronger seedlings than those of less vigorous ones. In most cases standard laboratory germination tests at optimum conditions may not necessarily indicate the seedling emergence performance of any lots in the field or glasshouse. Therefore, vigour tests were developed to test seed lot performance under a wide range of environmental conditions. Seed vigour tests of accelerated ageing, controlled deterioration and mean germination time were successfully used to estimate laboratory germination and seedling emergence in various crop seeds (Mavi and Demir, 2007; Mis et al., 2022). RE, the radicle emergence test, was introduced and validated by ISTA and used to predict not only laboratory germination (Mavi et al., 2016; Shinohara et al., 2021) but also seedling emergence in both modules and the field environment (Demir et al., 2008; Khajeh-Hosseini et al., 2009; Mavi et al., 2010; Matthews et al., 2011; Ermis et al., 2022), as well as seed longevity (Ermis et al., 2022; Eren et al., 2023). RE has various advantages compared to ageing tests, as it is easy to evaluate, faster and simpler, and is suitable for machine evaluation (Matthews et al., 2011). However, the RE test has rarely been used for landraces or genotypes that have a different genetic background (Demir et al., 2020). Faster and easier evaluation of seed germination and vigour potential of Luffa seed genotypes may help to decide faster and to select the right material for further breeding purposes as using for the next year's breeding programme. In this study, we tested whether RE can be used to estimate laboratory germination and seedling size in Luffa seed genotypes.

MATERIALS AND METHODS

Plant materials

Twelve seed lots of Luffa (*Luffa aegyptiaca* Mill.) genotypes were collected from various parts of Hatay, Turkiye (Mavi et al., 2018; Mavi et al., 2020). Seeds of genotypes from the S3 stage were used. They were harvested from mature yellow fruits. Seeds were dried at room temperature and kept at 5°C. Seeds were distinctively different in appearance and size. Six lots were black (1, 3, 5, 7, 10, 12), five (4, 6, 8, 9, 11) were brown, and one (2) was a white coated genotype. The weight of one hundred seeds varied between 10.4 g, lot 9, and 15.4 g, lot 2. Seed moisture content was between 6.7 and 7.3%.

Germination and emergence procedure

The germination of three replicates of 50 seeds from each seed lot was assessed using the between-paper method (ISTA, 2022) at 25ºC in the dark. Radicle emergence test counts (the number of seeds with a radicle ≥ 2 mm long) were made at 48, 72, 96, 120, 144 hours. At the final count, 14 days after the beginning of the test, seedlings were evaluated as total (TG) and normal germination (NG) percentages (ISTA, 2022). Mean germination time (MGT) was calculated on the daily radicle emergence counts by using the formula below:

$$\Sigma (n.t) / \Sigma n$$

where n is the number of seeds newly showing radicle emergence (>2 mm) at time t (hours). Σn is the total number of seeds showing radicle emergence by the end of the test.

The seedling emergence tests were conducted with three replicates of 25 seeds in each seed lot. The seeds were sown 4 cm deep in compost (Plantaflor, Humus Verkaufs, GmBH, Vechta, Germany) in sandwich boxes (360 x 220 x 60 mm), and all boxes were transferred to a growing cabinet for 16 days at 22±2 °C. Light was provided by cool fluorescent lamps (Philips, Hamburg, Germany) at a rate of 78 µmol m^2 s^{-1} for 12 h d^{-1} at the seedling level. The relative humidity in the cabinet was maintained above 65-70% throughout the experiment to minimize water loss from the boxes. The appearance of a hypocotyl hook on the compost surface was used as an emergence criterion, and emerged seedlings were recorded daily at the same time of the day. Seedling emergence (SE) percentages were determined 16 days after sowing. To determine seedling fresh weight, hypocotyl length and seedling length, destructive harvests were taken 16 days after sowing on 15 randomly seedlings (5 seedlings x 3 replicates) selected among normally developed seedlings of each genotype. Seedlings were taken out of the peat moss, cut just above the root and cleaned, and seedling fresh weight (SSW, 0.001 g/plant), and hypocotyl thickness (HT, 2 cm above the soil surface, mm/plant) were determined in each plant. The measurements were made within 10 seconds of destructive harvest.

Statistical analysis

Correlation and regression coefficients (R_2) were performed between radicle emergence test (RE) and total, normal germination percentages, mean germination time and seedling parameters by using SPSS (IBM version 25). Level of significance was determined as various significant levels.

RESULTS AND DISCUSSION

Final total germination (TG) of the seed lots ranged between 55 and 97%, and normal germination was between 53 and 93%. Statistically significant differences were determined between the genotypes in terms of the examined traits (Table 1). Cumulative RE percentages are shown in Figure 1. There are great range among the twelve seed lots starting by 24 hours towards 144 hours (Figure 1). The fastest germinating lots were lots 1 and 4. Both reached

97% over 144 hours. However, lots 10, 11 and 12 had the lowest values. They had lower than 65% germination over 144 hours. Lot 12 had just 47% germination over 144 hours. Seed lots had different MGT values of between 2.5 and 4.3 days, the lowest being in lot 10 and the highest in lot 1.

Table 1. Variation in total (TG, %), normal (NG, %), germination, mean germination time (MGT, d), seedling emergence (SE, %), Seedling shoot weight (SSW, g/plant), hypocotyl thickness (HT, mm/plant) of Luffa genotypes. Means with different letters in the same column are significantly different at the 5% level.

Seed Lot	TG	NG	MGT	SE	SSW	HT
1	97^{a}	93^{a}	2.5^{a}	100^{a}	1.21^{a}	2.61^{a}
2	97^{a}	92^{a}	3.0^{ab}	97^{a}	1.15^{ab}	2.44^{ab}
3	92^{ab}	90^{ab}	3.1^{ac}	73^{bc}	0.60^{d}	2.38^{ac}
4	93^{ab}	88^{ac}	3.3^{ad}	92^{a}	0.61^{d}	2.43^{ab}
5	92^{ab}	88^{ac}	3.8^{bd}	85^{ab}	0.57^{d}	2.42^{ab}
6	89^{ac}	87^{ac}	3.2^{ac}	82^{ab}	0.83^{cd}	2.30^{bc}
7	95^{ab}	86^{ac}	3.4^{ad}	88^{ab}	0.80^{cd}	2.43^{ab}
8	88^{ac}	80^{bd}	3.9^{bd}	50^{d}	0.74^{cd}	2.34^{ac}
9	84^{bd}	77^{cd}	3.3^{ad}	72^{bc}	0.56^{d}	2.40^{ac}
10	79^{cd}	69^{de}	4.3^{d}	48^{d}	0.90^{bc}	2.36^{ac}
11	76^{d}	64^{e}	3.6^{bd}	64^{cd}	0.93^{bc}	2.37^{ac}
12	55^{e}	53^{f}	4.1^{cd}	53^{d}	0.65^{cd}	2.13^{c}
Range	55-97	53-93	2.5-4.3	53-100	0.60-1.21	2.13-2.61

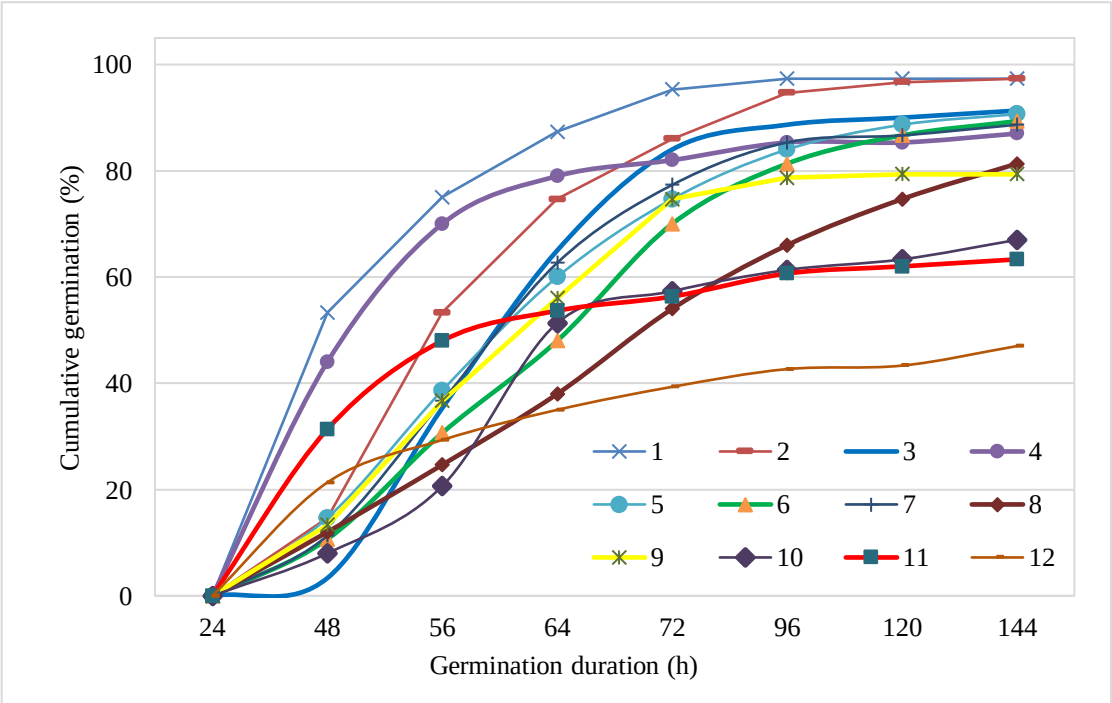

Figure 1. RE counts in the 144 hours after the germination test was set up in twelve Luffa genotypes.

Seed lot 1 had the highest seedling emergence percentages, the lowest mean emergence time, seedling shoot weight and hypocotyl thickness among all lots (Table 1). Lot 12 had 53% of seedling emergence and the thinnest of HT at 2.13 mm/plant.

The highest coefficient values between RE values and seed germination and seedling quality factors were found at 72, 96, 120 and 144 h (Table 2). We chose 72 hours for the sake of shorter evaluation time. RE72h was highly related to total ($P<0.001$, $R^2=0.732$) and normal ($P<0.001$, $R^2=0.751$) germination in laboratory conditions and MGT ($P<0.001$, $R^2=0.842$) values (Figure 2).

Table 2. Correlation coefficient values (r) for the relationship between RE and Total Germination (TG, %), Normal Germination, (NG, %), Mean Germination Time (MGT, day) Seedling emergence (SE %), Seedling Shoot weight (SSW, g/plant) and Hypocotyl Thickness (HT, mm/plant) in twelve Luffa genotypes. Significance: *: P<0.05,**: P<0.01,***: P<0.001.

RE hours	TG	NG	MGT	SE	SSW	HT
RE48	0.081	0.097	-0.371	0.424	0.422	0.454
RE56	0.446	0.467	-0.679*	0.762**	0.490	0.652**
RE64	0.736**	0.746**	-0.808**	0.793**	0.627*	0.836***
RE72	0.855***	0.866***	-0.917***	0.868***	0.591*	0.870***
RE96	0.937***	0.960***	-0.900***	0.872***	0.507	0.829***
RE120	0.959***	0.993***	-0.873**	0.827***	0.455	0.800**
RE144	0.971***	0.982***	-0.832***	0.768**	0.447	0.777**

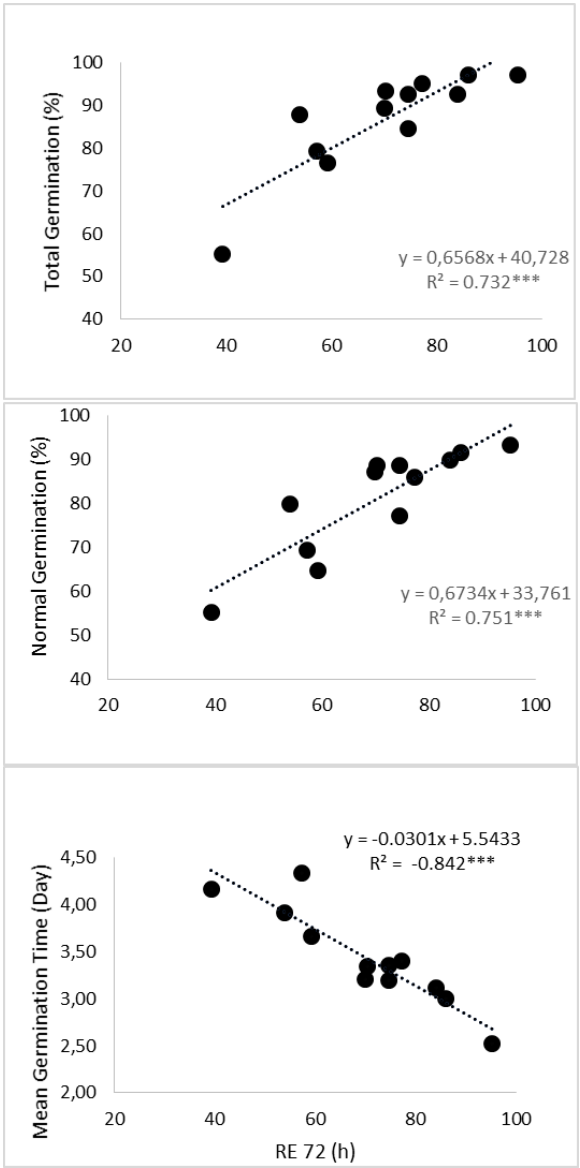

Figure 2. The relationship between RE72h and total (TG), normal (NG) germination percentages and mean germination time (MGT) in twelve Luffa genotypes. Significance ***: P<0.001).

The period in which the RE test showed the highest correlation with six different characteristics (72h) was determined as the most ideal period for the power test (Table 2). In previous studies, the highest correlation was determined in 48-hour RE tests in radish (Mavi et al., 2016) and 120-hour RE tests in carrot (Mis et al., 2022).

The results obtained in this study show that RE tests are successful at discriminating both the laboratory germination percentages (total /normal) in ideal germination conditions and seedling growth in modules, i.e. vigour of sponge gourd seed genotypes. Our work supports previous reports in which a single count of RE, identified from a germination progress curve, correlates with laboratory germination in many diverse crop seeds (Matthews and Khajeh-Hosseini, 2006; Khajeh-Hosseini et al., 2010; Matthews et al., 2018; Mavi et al., 2016; Powell and Mavi, 2016; Ozden et al., 2018; Demir et al., 2020; Mis et al., 2022). A single RE count after 48 hours at 13°C or 24 and 30 hours at 20°C in oilseed rape (Matthews et al., 2012), after 48 hours at 20°C in radish (Mavi et al., 2016) and cauliflower (Shinohara et al., 2021), and after 104 hours at both 25°C and alternating 20/30°C in aubergine (Ozden et al., 2018) is significantly related with NG. Work on onion seed and the application of an 80 hour RE count successfully predicted the NG of commercial lots (Demir et al., 2020): 40 h in lettuces, 74 h in watermelons and 120 h in carrots were found to be successful (Mis et al., 2022). The RE test has been also found to be succesful in discriminating lots according to seed vigour, i.e. emergence in modules and the field in several species (Demir et al., 2008; Khajeh-Hosseini et al., 2009; Mavi et al., 2010; Matthews et al., 2011, 2012; Ermis et al., 2022). The lower RE values were associated with longer time to germination as a consequence of seed deterioration (Matthews and Khajeh-Hosseini, 2006). This was proposed as the basis of the ageing-repair hypothesis and the basis of all vigour tests (Matthews et al., 2011).

The seed lots with lower RE count are the result of a longer lag period before radicle emergence begins (Matthews et al., 2012; Shinohara et al., 2021), which is due to the increased need to repair the deterioration caused by seed ageing. Various vigour tests can be used to discriminate differences in the level of ageing of seed lots, such as accelerated ageing (ISTA, 2022). The RE test is a valuable test for predicting vigour differences due to its simplicity and rapidity.

RE values showed a significant relationship with seedling quality parameters. The relations varied. RE72 was found to have a very highly predictive potential for seedling emergence ($P<0.001$, $R^2=0.754$), hypocotyl thickness ($P<0.001$, $R^2=0.757$) and seedling shoot weight in the seed lots (Figure 3).

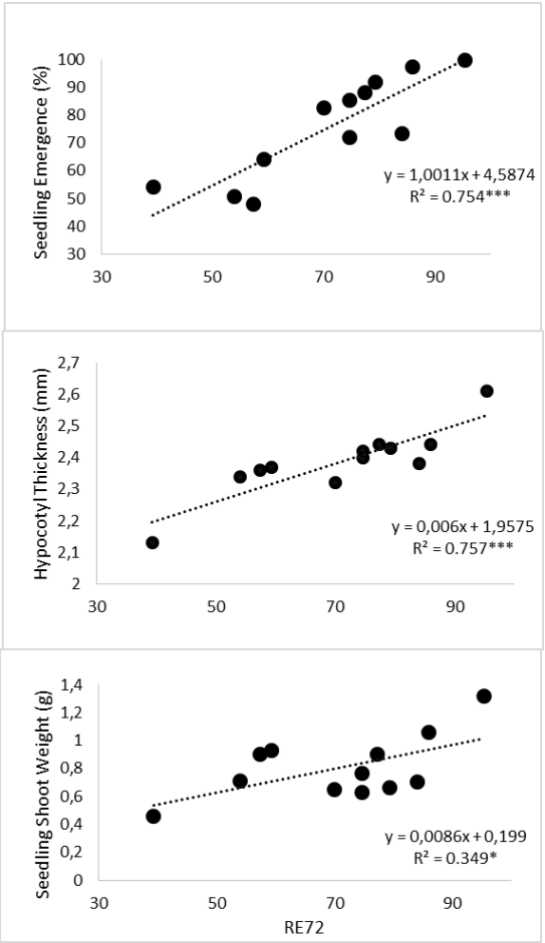

Figure 3. The relationship between RE72h and seedling emergence (SE), hypocotyl thickness (HT) and seedling shoot weight (SSW) in twelve Luffa genotypes. Significance *: P<0.05, ***: P<0.001).

Seed lots with higher RE values had higher total and normal germination and germination time but also had higher seedling quality parameters as reflected in seedling emergence, higher seedling shoot weight and hypocotyl thickness (Figure 3). RE results after 72 hours at 25°C show correlation with the results of seedling emergence percentages, seedling shoot weight and hypocotyl length. This suggests that the RE could be used to rank lots according to seedling emergence potential (Figure 3). Both criteria are important for grafted transplant production in cucurbits. Thicker hypocotyls provide a higher number of seedlings for graftable levels, which increase the efficiency of grafting. In this study, the seed lots were genotypes collected from various parts of the Hatay region. They were not genetically stable (homogeneous). For breeders, selecting higher quality seed lots is valuable. In our study, RE72 h indicates the higher total and normal germination of any seed lots very significantly (Figure 2). This can be a very helpful selecting method for breeders when a large number of seed lots are used in breeding programmes.

Sponge gourd is a heat-resistant cucurbit species, mainly used for grafting in cucumbers. Therefore, it is recommended to use sponge gourd as a rootstock for cucumber cultivation in high-temperature conditions. It has been reported that cucumbers grafted on to luffa rootstocks significantly alleviate growth reduction at high temperatures (Li et al., 2014). This study is valuable for determining the rootstock potential of the sponge gourd genotypes used and selecting a suitable rootstock genotype for this country. Particularly, genotypes with higher emergence percentages, seedling dry weight and hypocotyl thickness are considered to have potential rootstock characteristics (Figure 3). Sponge gourd genetic materials are very important both in the breeding of commercial varieties and in the breeding of rootstocks. For this reason, genotypes selected from Hatay should be evaluated both as vegetables and as rootstocks (Mavi et al., 2020). It has been determined that some of the genotypes can be evaluated as vegetables. Genotypes with high emergence and high hypocotyl thickness (Figure 3) can be used in experiments as rootstocks. When vegetable rootstock reach the graftable level in a shorter time, that is, they have higher values in the RE test, the efficiency of grafting can be higher. Fast germination results in thicker hypocotyls, and this makes it easier to do more grafting at one time, which helps to produce equally-sized transplants (Ermis et al., 2022).

CONCLUSION

In conclusion, the RE test was able to assess the seed germination percentages and seed vigour of seed genotypes in Luffa. The RE test has the advantages of being fast, easy and practical, and the analyst does not require the special training necessary for other vigour tests. Also, there is no use of chemicals. A single radicle emergence count after 72 hours at 25°C is therefore a successful alternative vigour test for Luffa seeds. This conclusion can be useful for ranking seed lots in relation to their germination and seedling emergence potential.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

All authors declare that they have no conflicts of interest.

Author contribution

Ahmet Hakan Eker (AHE), and Ibrahim Demir (ID) carried out experimental part of the study. Kazım Mavi (KM), and ID reviewed the manuscript, AHE done statistical analysis, KM and ID designed the experiments and ID conceived the principal idea and wrote the paper.

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Investigation of viruses and phytoplasma infections in tomato plantations in Bilecik province, Türkiye

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Abstract

In this study, the status of infections caused by viruses and phytoplasmas in tomato production areas of Bilecik province was determined using conventional molecular methods. During the 2022 tomato production season, 93 plants exhibiting symptoms such as mosaic, leaf and fruit deformations, flower anomalies, and necrotic spots were collected. Viral agents such as tomato spotted wilt virus (TSWV), cucumber mosaic virus (CMV), southern tomato virus (STV), tobamoviruses, and potyviruses were screened by RT-PCR, while phytoplasmas were detected by nested-PCR. Single, double, and triple infections were detected in 50 of the 93 plants. 17, 5, and 21 plants were infected by a single pathogen for TSWV, STV, and phytoplasmas, respectively. 1, 3, and 2 plants were infected by two pathogens for STV+TSWV, STV+phytoplasma, and TSWV+phytoplasma, respectively. Only one plant detected a triple infection caused by STV, TSWV, and phytoplasmas. The phytoplasma genetic group was determined as 16Sr XII-A by PCR RFLP *in-silico* and *in-vitro* methods. Sequencing studies revealed that TSWV had high nucleotide sequence similarity with other Türkiye isolates for the NSs partial gene and STV entire CP gene region. For phytoplasmas, sequencing studies showed that the obtained tomato strains overlapped one-to-one with stolbur strains. Phylogenetic analyses applied with global isolates for TSWV NSs and STV CP gene regions showed the existence of 2 main groups (Clade I and Clade II). TSWV and STV isolates obtained from this study clustered in large main branches (Clade I).

Keywords: Bilecik, Tomato, Virus, Phytoplasma, PCR, Sequencing

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the most widely grown and consumed vegetables. It has a very high climate tolerance and is produced in different systems such as open field, hydroponic, polytunnel, and greenhouse. Currently, tomato production amounts is 180 million tons globally. Türkiye, which has a wide climatic zone, is one of the leading countries with an average annual yield of 13 million tons. In Bilecik Province, located in the South Marmara region and the transitional climate zone, it is recorded that an average of 107.000 tons of fresh and paste tomatoes are produced in an area of approximately 16,000 da. In the region, tomato production is quite common due to the flavour of local tomato varieties and the efficient use of agricultural areas.

It is well known that many phytopathogens cause significant yield and quality losses in tomato cultivation. In particular, viral infections can lead to decreased fruit quality and yield, reduced nutrient content and shorter shelf life, making tomatoes unmarketable (Hanson et al., 2016). Over 130 viruses and virus-like agents have been reported to cause infections in tomato plants. More specifically, viruses of different species have been reported as significant agents in tomato production areas from many countries in the Mediterranean basin, causing substantial

yield and quality loss. These viruses are tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), tomato infectious chlorosis virus (TICV), cucumber mosaic virus (CMV), tomato chlorosis virus (ToCV), alfalfa mosaic virus (AMV), and pepino mosaic virus (PepMV) have been recorded as agents that cause established and chronic infections (Panno et al., 2021; Roselló et al., 1996; Navas-Castillo et al., 2011). Recently, there have been reports of the emergence of viruses that cause serious restrictions in tomato production, including torradoviruses and tomato brown rugose fruit virus (ToBRFV) (Van der Vlugt et al., 2015; Oladokun et al., 2019). The presence of viral agents such as AMV, TSWV, CMV, southern tomato virus (STV, Amalgavirus), ToBRFV, ToCV, and TYLCV has been reported in tomato production areas located in different geographical regions of Türkiye (ustaeşilyurt and Çevik, 2019; Fidan et al., 2019; Güller and Usta, 2020; Karanfil, 2021; Akdura and Çulal Kılıç, 2022; Randa-Zelyüt et al., 2023; Usta et al., 2023; Güller et al., 2023).

Another pathogen that causes significant infections in tomato is phytoplasma of the phylum Firmicutes (class Mollicutes), a group of cell wall-less microorganisms genetically related to a Gram-positive ancestor (Weisburg et al. 1989). It is well known that various genetic groups of phytoplasmas, which are limited to the phloem tissue in plants and transmitted by insect vectors, cause different or similar symptoms in many agricultural products grown in many parts of the world (Kumari et al. 2019; Hogenhout et al.,2 008). Specifically, '*Candidatus* Phytoplasma solani' (CaPsol) (subgroup 16SrXII-A), which is highly prevalent in Euro-Mediterranean grapevine agroecosystems and a potential threat to viticulture worldwide, has also been reported to cause significant infections in Solanaceae and Apiaceae members (Navrátil et al. 2009; Quaglino et al. 2019; Ember et al., 2016). In Türkiye, it has been reported that CaPsol causes infections in various perennial and annual agricultural products, especially tomatoes (Güller and Usta, 2020; Çağlayan, 2023; Randa-Zelyüt et al., 2022; Randa-Zelyüt, 2023).

This study aimed to determine the possible viral and phytoplasma infections that cause yield and quality loss in products and cannot be controlled by direct chemical control methods in the regions of Bilecik province of Marmara region where tomato production is intensive. The presence of pathogens was determined by conventional molecular methods and molecular characterization of genes of viruses and conserved 16Sr RNA gene regions of phytoplasmas were genetically identified.

MATERIALS AND METHODS

Field studies

Between June and September of 2022, field surveys were done in open fields and plastic tunnels where tomato cultivation was carried out in the province of Bilecik, Türkiye. A total of 93 tomato plants with symptoms caused by viruses and virus-like agents were collected from İnhisar, Osmaneli, Söğüt, Gölpazarı, and Merkez Districts. The samples were brought to the laboratory, labelled and stored at -20 ℃ for molecular studies. A symptomatic description of the samples was recorded

Total Nucleic Acid (TNA) Isolation and cDNA synthesis

CTAB-based Total Nucleic Acid (TNA) isolation method with minor modifications (Li et al. 2008) was applied to ± 150 – 160 mg epidermal and vascular tissues of tomato leaves since the molecular study used both RNA and DNA molecules as PCR templates. cDNA libraries were constructed to detect RNA viruses considered within the scope of this study. In the first step, 3 µL RNA was denatured with 0.2 µg/µL Random Hexamer primer (5'-NNNNNNNN-3') (Thermo Sci., USA) and DEPC-water (Thermo Sci., USA) to a volume of 12.5 µL at 95 °C for 3 minutes and the tubes were denatured at 95 °C for 3 minutes and they were kept on ice for 5 minutes. In the second step, 10 µL of enzyme-containing 100 units of reverse transcriptase enzyme (RevertAid Reverse Transcriptase 200 U/µL, Thermo Sci., USA), 20 units of Rnasin (RiboLock RNase Inhibitor 40 U/µL, Thermo Sci., USA), 2 µL (10 mM) dNTP, 1X RT-buffer (250 mM Tris-HCl (pH 8. 3 at 25°C), 250 mM Tris-HCl (pH 8.3 at 25°C), 250 mM KCl, 20 mM MgCl2, 50 mM DTT) and enzyme mixture containing 1.25 DEPC-water were prepared and added to the tubes on ice. The tubes were incubated at 25°C for 15 min (random primer binding), 42°C for 60 min (enzyme forming the templates) and 4°C for 5 min. The cDNA libraries obtained were stored at -20 °C for use in amplification studies.

Amplification Assays

The presence of CMV, TSWV, tobamoviruses, potyviruses, and STV in tomato plants was investigated in cDNAs obtained. Therefore, molecular detection studies were applied for the detection of specific gene regions of the aforementioned viruses, both species- and genus-specific. For amplifications, 2 µL cDNA, 1X buffer, 2 µL (25 mM) $MgCl_2$, 0.5 µL (forward and reverse; 10 µM) primers, 0.5 µL dNTP (10 µM), and 1.25 units of Taq enzyme (Thermo Sci., USA) were used in a total reaction volume of 20 µL. Details of the primer sequences used for virus detection are given in Table 1.

TNAs obtained in the molecular detection of phytoplasmas were diluted 1:30 with nuclease-free water- NFW (Invitrogen, USA) and then used in nested-PCR studies as 1 µL gDNA template in a total volume of 20 µL. For the first step reaction, 1 µL gDNA, 1X buffer, 2 µL (25 mM) MgCl2, 0.5 µL dNTP (10 µM), 1.25 units of taq enzyme, and 0.5 µL (10 µM) each primer were used in a total volume of 20 µL. For the second step of PCR studies, the first amplification products were diluted 1:30 and reacted with R16F2n and R16R2 universal phytoplasma

primers under the same reaction conditions stated above. Details of the primer sequences used for phytoplasma detection are given in Table 1.

Table 1. Primer sequences used in the detection of viruses and phytoplasmas by PCR methods

The causal agent	Primer name	5'-3' sequence	Size and gene region	Reference
TSVW	NSS2-F	GCTTCAGTCTGGGGATCAACT	724 bp Partial non-structural (NSs)	Abadkhah et al. 2018
	NSS2-R	TTGGAACTCTTAGCCAGAGGC		
Tobamovirus	TobamodF	TKGAYGGNGTBCCNGGNTGYGG	880 bp Partial RdRp	Li et al., 2018
	TobamodR	ACNGAVTBNABCTGTAATTGCT AT		
CMV	CMV_CPF	ATGGACAAATCTGAATCAACC	638 bp Partial CP	Karanfil and Korkmaz, 2017
	CMV_CPR	GATGTGGGAATGCGTTGGTGC		
Potyvirus	Potyvirus-F	GTITGYGTIGAYGAYTTYAAYAA	350 bp Partial Nib	Zheng et al. 2008
	Potyvirus-R	TCIACIACIGTIGAIGGYTGNCC		
STV	STV_ CPF	CTCGTCGTTGCTTCCGTT	1134 bp Complete CP	Randa-Zelyüt et al. 2023
	STV_ CPR	ACCACCACCCCTGTACTT		
Phytoplasma	R16mF2	CATGCAAGTCGAACGGA	1.8 kb Partial 16Sr RNA	Gundersen and Lee, 1996
	R16mR2	CTTAACCCCAATCATCGA		
	R16F2n	GAAACGACTGCTAAGACTGG	1.2 kb Partial 16Sr RNA	Lee et al. 1993
	R16R2	TGACGGGCGGTGTGTACAAACC CCG		

Determination of 16s rRNA Genetic Groups of Phytoplasmas by *in-vitro* and *in-silico* PCR-RFLP Methods

To determine the genetic group of phytoplasmas according to the 16s rRNA region, digestion profiles were prepared using both in-vitro and in-silico methods. For this purpose, PCR-RFLP (Restriction Fragment Length Polymorphism) profiling of the 1.2 kb PCR products obtained with R16F2n/R16R2 primers was performed under in vitro conditions using the AluI endonuclease enzyme. A standard reaction protocol was used for polymorphic profiling. For AluI enzyme (Eurx, Estonia), 5 µL PCR product (≈ 0.1-2 µg), 2.5 µL of 10 X reaction buffer, 0.25 µL of 100 X BSA (Bovine serum albumin) (Eurx, Estonia), 6 units of enzyme and the remaining portion was completed with nuclease-free water in 25 µL volume. The mixture was incubated at 37 0C overnight for at least 16 hours to allow the reaction to take place. The products were then run on a 1.8% agarose gel in 1X TAE buffer solution at 80 V for 90 min. UV device (Syngene, UK) was used for visualisation. After sequence analyses of some R16F2n/R16R2-amplified products selected according to the profiles were completed, computer-simulated in silico PCR-RFLP cutting was performed using iPhyClassifier (Zhao et al. 2013) software.

Sequencing and Phylogenetic Inferences

For sequencing, selections were made based on the regions where infection was detected from the fragments obtained as a result of amplification studies. To extract the nucleotide sequences of these fragments, sequencing was performed by outsourcing services (BM Labosis, Ankara). After the raw nucleotide data of the isolates obtained from viruses and the strains obtained from phytoplasmas were edited in Bioedit software, the studies were continued in MEGA X (Kumar et al., 2018) software. Sequences of relevant global isolates were extracted from GenBank and data sets were prepared. Alignment of relevant gene regions was performed using the ClustalW algorithm. Phylogenetic analyses were applied using the Tamura-3 (T-92) parameter and the Neigbour-Joining method. Additionally, iTOL software was used to visualize the trees. In addition, the similarity ratios of the obtained nucleotide and amino acid sequences were calculated in SDT software.

RESULTS AND DISCUSSION

Symptoms such as chlorosis, necrosis, mosaic, yellowing, embrittlement, epidermal tissue with narrowed vein angles, weak leaf formations, deformations, reddening of veins and proliferation were observed on the leaves of tomato samples during field studies (Figure 1a and 1b). Dead and unpollinated flower structures were observed in the plant's flowers (Figure 1c and 1d). In the fruit, symptoms such as severe deformation, necrosis, round spots, fungal and cracked tissues, and small weak fruit formations were observed (Figure 2a-d).

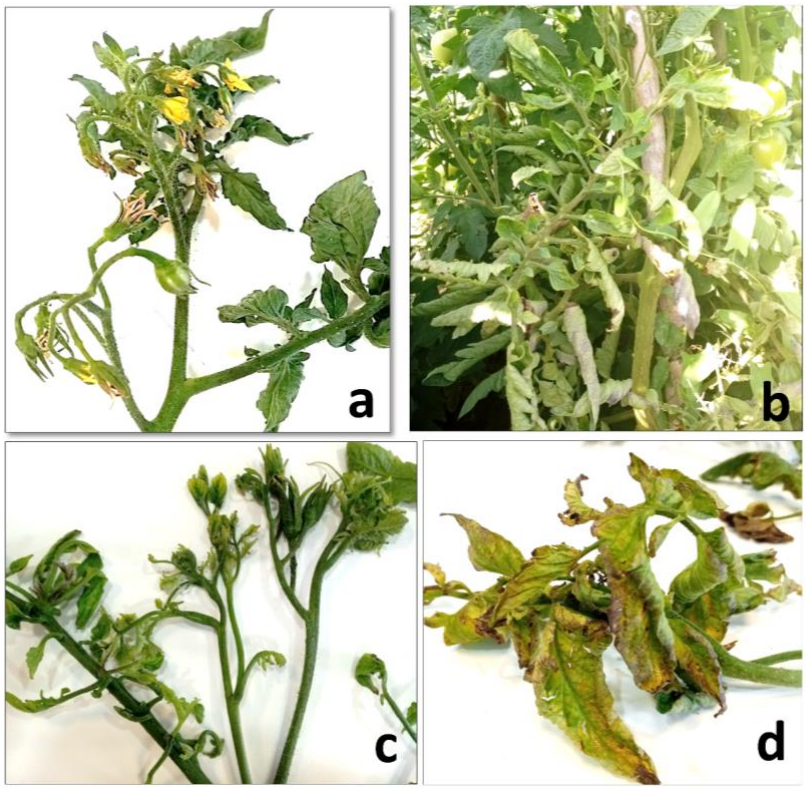

Figure 1. Malformation and death of tomato flowers (a-c). Curling and necrotic spots on leaves (b-d).

Figure 2. Deformation in tomato fruits, yellow ring spots, brown and necrotic sunken areas (a-d).

PCR study revealed fragments of the expected size for STV in 10 samples and TSWV in 21 samples. CMV, potyvirus and tobamovirus agents were not detected in all 5 districts by the molecular method. Fragments of 1134 bp, corresponding to the fully encoded CP gene region for STV, and 774 bp, corresponding to the complete encoded NSs gene region for TSWV, were obtained. For phytoplasmas, 1.25 kb fragments corresponding to a part of the 16Sr gene region were obtained with universal primer sets in 27 tomato samples. these infections were

distributed as single, double and triple in the samples. However, virus and phytoplasma infections were not detected in the Merkez and Gölpazarı districts. Single infection was detected in 17, 5, and 21 samples for TSWV, STV, and phytoplasmas, respectively. STV and TSWV together caused double infections in 1 sample, STV and phytoplasma in 3 samples, and TSWV and phytoplasma in 2 samples. Finally, a triple infection caused by STV, TSWV, and phytoplasmas was detected in 1 sample. Infection rates are detailed in Table 2.

Table 2. Regional distribution and rates of infections

Region	Number of samples	TSWV	STV	16Sr	STV + TSWV	STV + 16Sr	TSWV + 16Sr	STV + TSWV + 16Sr
İnhisar	22	11 (11.8%)	3 (3.2%)	4 (4.3%)	1 (1.1%)	2 (2.2%)	2 (2.2%)	-
Osmaneli	34	-	2 (2.2%)	13 (13.9%)	-	1 (1.1%)	-	-
Söğüt	29	6 (6.5%)	-	4 (4.3%)	-	-	-	1 (1.1%)
Merkez	3	-	-	-	-	-	-	-
Gölpazarı	5	-	-	-	-	-	-	-
Total	93	17 (18.3%)	5 (5.4%)	21 (22.3%)	1 (1.1%)	3 (3.2%)	2 (2.2%)	1 (1.1%)

Virus and phytoplasma diseases are major obstacles in the cultivation of horticultural products worldwide that may lead to significant yield losses. The findings of this study based on molecular detection methods showed that TSWV, STV and CaPsol agents caused damage in tomato plants in the districts of Bilecik province.

The molecular test applied in this study confirmed the presence of TSWV and STV in Söğüt and İnhisar districts as well as phytoplasma in Söğüt, İnhisar, and Osmaneli districts of Bilecik province. TSWV infection rate was especially high in İnhisar where 14 of 22 (64%) of the samples tested positive for the virus. However, infections caused by TSWV have been reported from different geographical regions of Türkiye and a wide variety of hosts (Bozdoğan and Kamberoglu 2016; Morca et al. 2022; Güneş et al. 2022; Usta et al., 2023; Sajid and Elçi, 2024). Severe symptoms (fruit deformations) were observed in the fruits of TSWV-infected plants during field observations, and similar symptoms were also reported by Morca et al. (2022). Furthermore, our study revealed that TSWV-infected plants exhibited leaf symptoms like severe yellowing, necrosis, and brown spots, which have been documented in various parts of Türkiye (Sajid and Elçi, 2024). Vector control may need to be upped in Bilecik. It is also worth noting that infection rates of phytoplasma were much higher than viruses in Osmaneli which may be related to the practice of using varieties resistant to the viruses there. Only a handful of samples were collected from Merkez and Gölpazarı which are not tomato cultivation regions, and the samples were all free from the tested causal agents. Furthermore, infections caused by CaPsol in both tomatoes and other agricultural products have been reported from many different geographical regions of Türkiye (Usta et al. 2018; Güller and Usta, 2020; Usta et al. 2021; Çağlar and Şimşek, 2022; Randa-Zelyüt et al. 2022). Especially in tomatoes, symptoms such as leaf curling, floral distortion and growth retardation have been observed (Usta et al. 2021; Çağlar and Şimşek, 2022; Zelyüt, 2023). Virus and phytoplasma vectors might not widely disperse and thus easier to manage in the two districts than Söğüt, İnhisar, and Osmaneli districts.

Tomato samples infected with TSWV, STV, and phytoplasma were selected to represent each region to perform their molecular characterization. Thus, 7 TSWV, 5 STV isolates and 6 phytoplasma strains were selected and their nucleotide sequences were obtained for the NSs, CP and 16Sr RNA gene regions, respectively. After the obtained nt sequences were edited in MEGAX software, BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analyzes were performed. The partial NSs gene region of 7 TSWV isolates obtained from Bilecik province showed more than 99.8% nt identity with another isolate (MK922155) obtained from Türkiye. The CP gene region of 5 STV isolates showed more than 99.9% nt identity with other isolates obtained from Türkiye. The 16Sr RNA gene region of 6 phytoplasma strains showed 99-100% nt similarity with other '*Candidatus* Phytoplasma solani' strains. Nucleotide sequence information of these isolates and strains were deposited in GenBank and accession numbers were obtained (Table 3).

Table 3. GenBank accession numbers of virus isolates and phytoplasma strains

TSWV		STV		16Sr XII-A	
Accession no	Isolate	Accession no	Isolate	Accession no	Strain
PP842680	Sogut1	PP836129	Bilecik 1	PP837564	Sogut1
PP842681	Sogut2	PP836130	Bilecik 2	PP837565	Sogut2
PP842682	Sogut3	PP836131	Bilecik 3	PP837566	Osmaneli1
PP842683	Sogut4	PP836132	Bilecik 4	PP837567	Osmaneli2
PP842684	Inhisar1	PP836133	Bilecik 5	PP837568	Inhisar1
PP842685	Inhisar2	-	-	PP837569	Inhisar2
PP842686	Inhisar3	-	-	-	-

The partial NSs gene region of the TSWV agent isolates obtained from this study (N = 7) and in the GenBank (N = 92) was used for phylogenetic analyses. A total of 99 isolates were clustered into 2 major branches Clade I and Clade II (Figure 3). The isolates obtained in this study were grouped in the largest main branch Clade I only with isolates obtained in Türkiye.

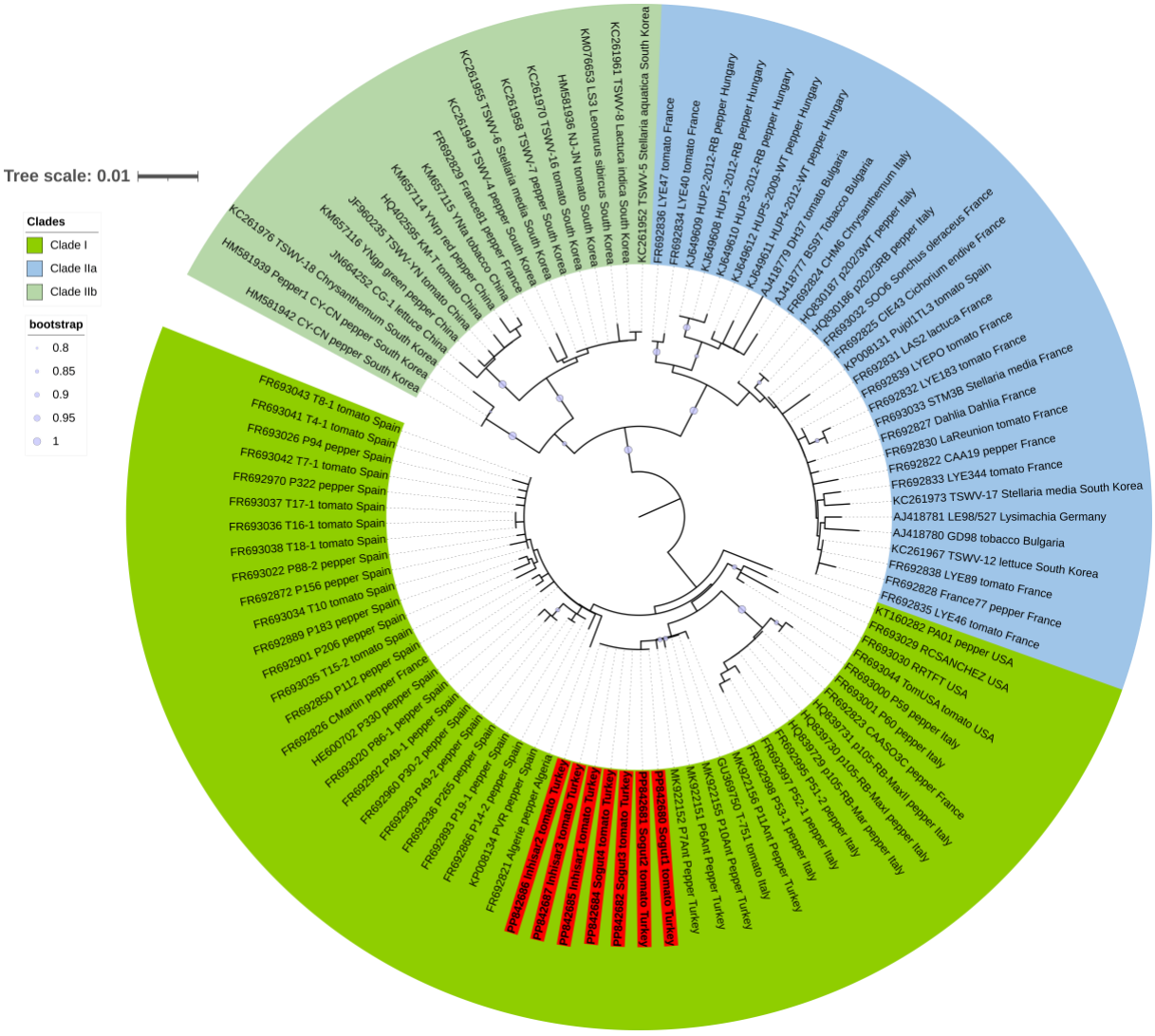

Figure 3. Dendrogram obtained as a result of the phylogenetic analysis applied for the partial TSWV NSs gene. Clusters of isolates from this study are marked in blue. Tamura-3 (T-92) algorithm was created with the Neighbor Joining (NJ) method and no outgroup was used.

For the STV CP gene region, global isolates (N=107) and isolates obtained in this study (N=5) were divided into two main branches Clade I and Clade II, and Turkish isolates were collected in Clade I (Figure 4). Complete CP region of STV isolates from regions of Türkiye had been sequenced, and they are positioned in distant subclusters within Clade 1 together with isolates from other countries in the constructed phylogenetic tree. PP836130 and PP836131 genetically were shown to have the closest relationship with isolates from Bursa while PP836132 and PP836133 with isolates collected in Balıkesir and Çanakkale Provinces which all are geographically proximate to Bilecik. Interestingly, PP836129 were related closely to Slovenia and Spain isolates. The results indicated that STV populations in Bilecik as well as the whole of Türkiye are highly stable as reported in previous studies (Randa-Zelyüt et al. 2023).

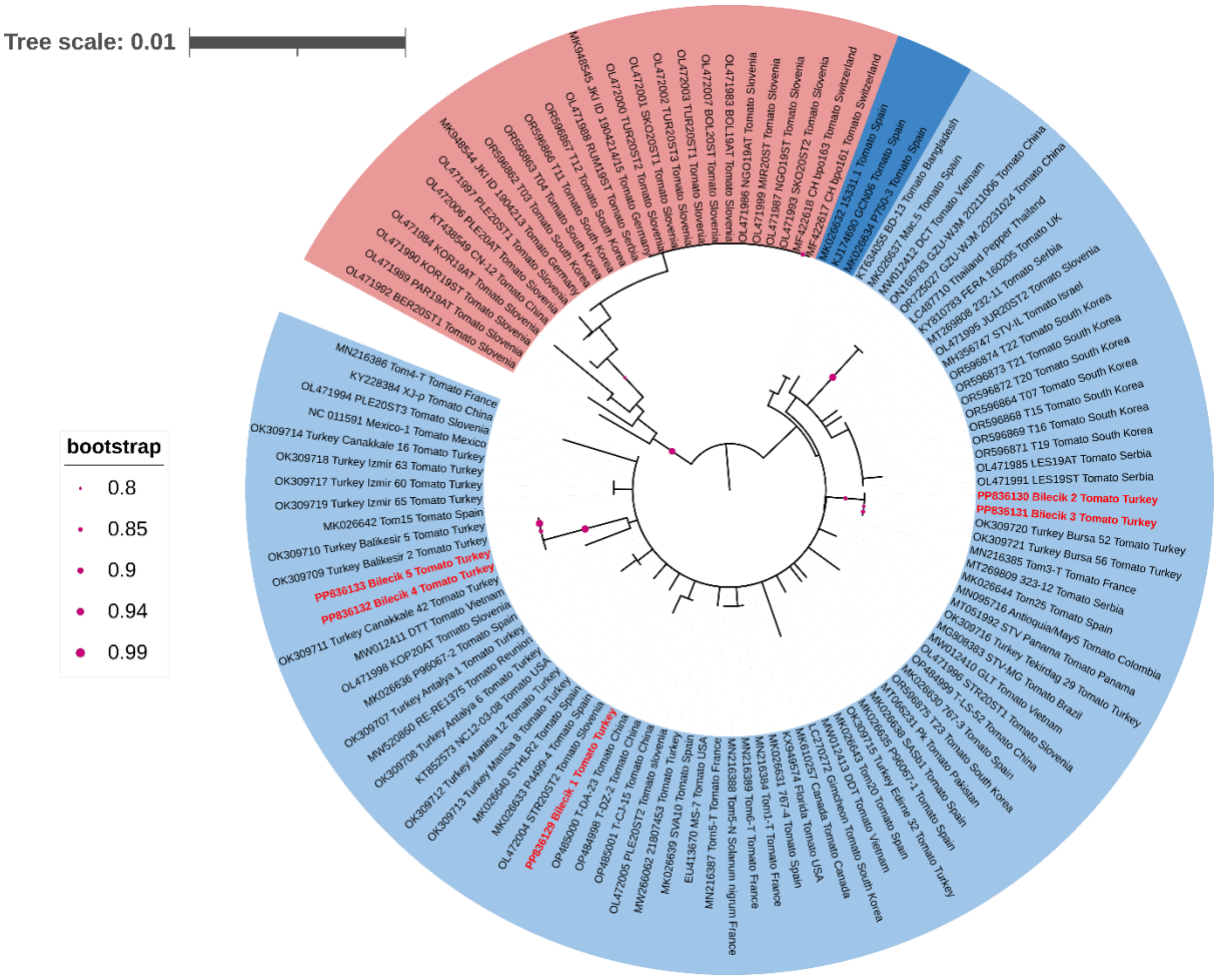

Figure 4. Tree created according to the fully encoded CP gene region of the STV. Clusters of isolates from this study are marked in light blue and the isolates were shown in red. Tamura-3 (T-92) algorithm was created with the Neighbor Joining (NJ) method and no outgroup was used. The blue pattern indicates Clade I and the other color indicates Clade II.

Amplicons obtained from 27 phytoplasma-infected tomato plants, 1.2 kb in size, as a result of nested-PCR studies, were digested with *Alu*I endonuclease under *in-vitro* conditions. The digest products were run on agarose gel with reference isolates and general profiles were obtained (Figure 5). To better understand the profiles, the '*Ca*. P. asteris' related strain Cabbage chloranthy (CHLL) (16Sr I-B) (France, INRAE) strain was used as a reference for comparisons. The digesting profiles obtained with *Alu*I exhibited the same profiles as the 16SrI-B '*Ca*. P. asteris' reference.

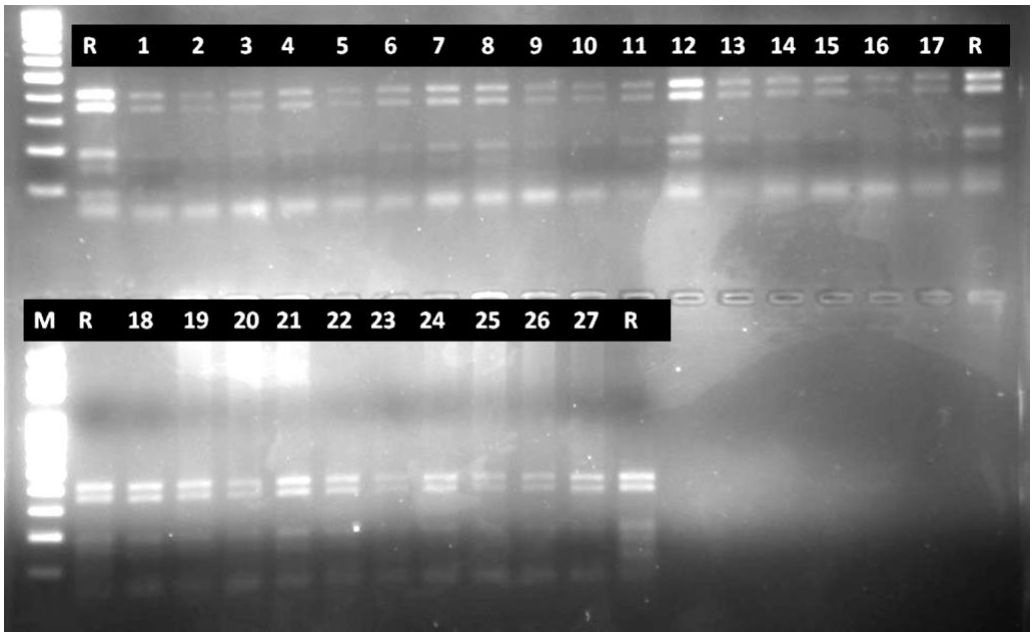

Figure 5. *Alu*I enzyme digesting profiles of fragments obtained with R16F2n / R16R2 primers of phytoplasma infected samples (R: Reference, CHLL Aster yellows 1-27 tomato samples from Bilecik province).

The nt sequences of 6 Bilecik tomato strains selected from these profiles were obtained. These sequences were analyzed using the *iPhyclassifier* software and subjected to in-silico digesting, and each strain was checked for group and subgroup verification in the same software (Figure 6). The software revealed that tomato phytoplasma strains obtained from Bilecik province shared more than 99% sequence identity with the '*Ca*. P. solani' 16Sr XII-A genetic subgroup.

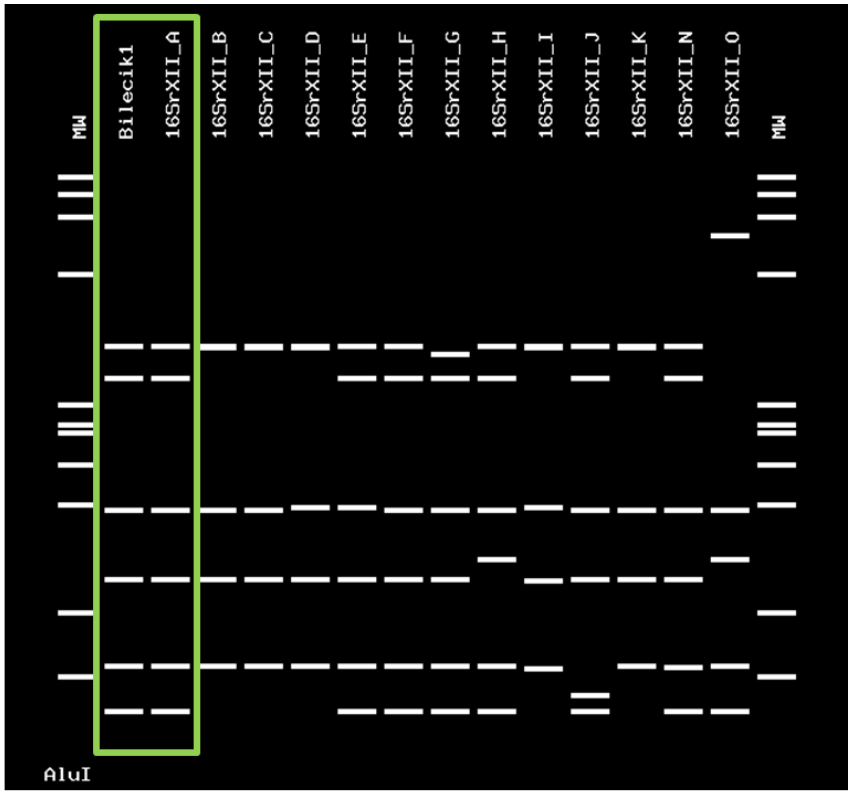

Figure 6. Comparison of 16Sr XII subgroups and *Alu*I endonuclease digesting profiles of 16Sr XII-A reference tomato sample from Bilecik province. The green rectangle shows the pattern of 16Sr XII-A and Bilecik province strain (Sogut1).

Phytoplasma infecting tomato in Bilecik was identified to belong to '*Ca*. P. asteris' 16Sr I-B subgroup due to band patterns after digestion of PCR amplicons using *Alu*I endonuclease *in vitro* analysis. However, *in silico* analysis using iPhyClassifier software suggested that the strains were '*Ca*. P. solani' 16Sr XII-A genetic subgroup. The study confirmed that phytoplasma is increasingly understood as a major pathogen of tomato in Türkiye, and sufficient control, probably through its vector management, needs to be implemented to avoid further damage.

CONCLUSION

The most important method for controlling viral agents is the breeding of resistant varieties. Breeding programs aimed at TSWV in tomato plants in particular are ongoing. However, varieties developed according to the host resistance genes are exposed to the molecular mechanisms of the virus that break the resistance over time. More efforts should be made to understand these mechanisms and genetic characterization studies should be carried out on different gene regions of the virus. However, the global seed trade impacts the spread of viral agents and affects the prevalence of STV. In addition, in controlling CaPsol phytoplasma, which has a very wide host range, it is important to ensure control of vector insects and weeds in cultivation areas.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

Conceptualization: F.R.Z, Methodology: A.K., A.I.S. and F.R-Z., Investigation: A.K. and F.R-Z Formal analysis: A.I.S., A.K., and F.R-Z., Writing—original draft preparation: A.K., F.R.Z., and A.I.S., Writing—review and editing:F.R-Z., Project administration: F.R.Z. All authors have read and agreed to the published version of the manuscript.

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Assessing the potential of mosquito larval rearing water for enhanced tomato seedling establishment

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Abstract

Vigorous seedlings guarantee satisfactory production in the forward stages of the vegetation period. This study aimed to evaluate the impact of bio-based rearing water of two mosquito species (*Culiseta* sp. and *Culex* sp.) on tomato germination, emergence, and seedling quality. For this purpose, two distinct larval-rearing waters (LRW)(with diverse larval densities), and fry food-applied water were used as bio-priming agents. The findings revealed that using bio-based rearing water could enhance the vigor of tomato seeds. All *Culex* sp. derived LRWs had a shorter mean germination time than the control group. One *Culex* sp. derived larval rearing water treatment resulted in the shortest mean germination time (4.35 days), whereas one *Culiseta* sp. derived larval rearing water treatment resulted in the longest (6.20 days). There were no statistically significant differences in stem length but significant differences in plant length. Plant length was shorter in larval rearing water and fry food-applied water than in the control. The stem diameters of plants primed with larval rearing water were generally wider than the control. According to analyses of the plant length, stem length, and stem diameter measurements, the larval rearing water and fry food-applied water treatments may have had a reductive influence on plant length but provided significant support for thicker seedlings, which are more beneficial for seedlings. Other germination and growth characteristics (vigor index of germination, emergence percentage, mean time of emergence, vigor index of emergence, plant length, stem length, leaf width, leaf length, stem fresh weight, stem dry weight, root dry weight) did not show significant variation among treatments. Using larval rearing water as a bio-priming agent in agriculture offers several benefits. Larval rearing water enhances seed germination and vigor due to its possibly rich nutrient content and bioactive compounds, promoting faster and more uniform germination. It is an eco-friendly and cost-effective alternative to chemical treatments, supporting sustainable agricultural practices.

Keywords: Priming, Tomato, Seedling, *Culex* sp., *Culiseta* sp.

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INTRODUCTION

Tomato is the most important and producing crop among vegetables in the world. In 2021, just 189 million tons of tomatoes were harvested in a 5 million ha (FAOSTAT, 2023). Using seedling in tomato production is commonly practices. Satisfactory plant growth and high yield can only obtain with healthy, uniform and vigour seedlings (Çelebi, 2019). In seedling production, a high emergence rate and healthy and homogeneous seedlings in a short time is very important (Arın & Arabacı, 2019). Therefore, some pre-sowing seed treatments are widely used to improve seed quality. One of them is priming which initiate the physiological process of germination with limited water intake under controlled conditions of seeds before sowing, to obtain high and homogeneous seed germination and emergence. Various compounds or treatments, such as salts, chemical solutions, bio compounds, etc., can be used for this purpose (Alan & İlbi, 2018; Azarmi et al., 2011; Farooq et al., 2005; Hernández et al.,

2021; Hernández-Herrera et al., 2014; Li et al., 2023; Nakaune et al., 2012; Nawaz et al., 2011). Using bio-based or organic compounds for such applications may be more environmentally and safer than chemical compounds (Chakraborti et al., 2022; Righini et al., 2021). Other hand, recycling biological wastes can reduce environmental pollution and convert waste into valuable agricultural inputs. This recycling process contributes to sustainability by promoting a circular economy, enhancing resource efficiency, and reducing the reliance on synthetic chemicals. These applications not only increase the vigor of seedlings but also can accelerate germination and provide resistance to unfavorable environmental conditions, such as extreme temperatures or humidity (Singh et al., 2020; Toribio et al., 2021).

Mosquitoes belonging to the *Culex* spp. family are prevalent and can be found in tropical and temperate climate zones across all continents except Antarctica (Diaz-Badillo et al., 2011). *Culiseta* sp. larvae are commonly found in rock pools or artificial containers, often associated with *Culex* sp. However, seeing them in natural water bodies such as pools or ditches is rare (Becker et al., 2020). Only a few studies have been undertaken to investigate the effects of mosquito larvae on rearing water, focusing on the problem from a microbiological standpoint. These studies revealed that the presence of mosquito larvae can alter the microorganismal composition of water and can increase, decrease, or have no effect on total bacterial abundance depending on the species and environmental conditions (Kaufman et al., 1999; Muturi et al., 2020; Paradise & Dunson, 1998; Walker et al., 1991). Most relevant studies have concentrated on a few mosquito species, including *Aedes triseriatus* (Kaufman et al., 1999; Paradise & Dunson, 1998; Walker et al., 1991), *Culex nigripalpus* (Duguma et al., 2017), and *Culex restuans* (Muturi et al., 2020). The trophic cascade has been used to describe the impacts of larvae on rearing water (Duguma et al., 2017; Muturi et al., 2020). However, it has been underlined that mosquito larvae can play a vital role in structuring microbial food sources, and the larvae's release of some nitrogenous wastes may be one of the critical consequences of this phenomenon (Muturi et al., 2020).

In our previous study, we saw that priming with LRW of *Culex pipiens* improved germination, emergence of cabbage seeds and seedling quality (Şahin et al., 2022). This time, we have evaluated the effect of bio-based larval rearing water of mosquito species Culiseta sp., and Culex sp. on germination and emergence of tomato seeds and also, on seedling characteristics.

MATERIALS AND METHODS

The experiments were conducted using UGT-281824 tomato seeds supplied by The United Genetics Seed Company. In the study, two distinct larval-rearing waters (LRW) and fry food-applied water (FFW) were used as agents, while distilled water served as the control (C). The first LRW was derived from water containers including *Culiseta sp.* with five distinct densities (Cs 1; 1 instar larva, Cs 3; 3 instar larva, Cs 4; 4 instar larva, Cs 7; 7 instar larva, and Cs 8; 8 instar larva), and the second LRW was derived from water containers including *Culex sp.* with seven distinct densities (4X; 4 instar larva, 8X; 8 instar larva, 16X; 16 instar larva, 32X; 32 instar larva, 64X; 64 instar larva, 128X; 128 instar larva, and 256X; 256 instar larva) of the first instar larva.

The germination tests were conducted for ten days, in a Plant Growth Chamber (ALC 800) set to 25±1°C, 65±5 % RH, and complete darkness. Fifty seeds were planted in each 9 cm ∅ plexiglass Petri dish, and 5 ml of LRW were used for moistening (distilled water for control). When radicle emergence was ≥2mm, it was considered to be a germinated seed and counted daily. The emergence tests were carried out on growth shelves equipped with daylight fluorescents. Seeds were sown in 72 cell seedling trays with 0.1 ml volume. As a growing medium, peat recommended for vegetable seedling production was used (Klassmann Potground-H, DoktorTarsa Inc., Antalya, Türkiye). It possessed a pH of 6.0 and an EC-value of 0.40 dS m^{-1} and added amount of fertiliser (NPK fertiliser 14:10:18): 1.5 kg m^{-3}.When the emergence began, seedlings were exposed to 195-210 µmol $m^{-2}s^{-1}$ PPFD (photosynthetic photon flux density) for 14 h/day. The counts were done every day and the seedling with fully opened cotyledons were recorded as emergenced. During the emergence test, average minimum and maximum relative humidity (RH%) and temperature (°C) values were recorded as 48 RH%, 54 RH%, and 21°C, 27°C, respectively.

The germination (GP) and emergence (EP) percentages were transformed arcsine square root before statistical analyses, but real values of germination/emergence percentages were presented in Tables. Mean germination time (MT_G) and mean emergence time (MT_E) were calculated according to Equation 1. Vigor indexes of germination (VI_G) and emergence (VI_E) were calculated according to (Mereddy et al.) (Equation 2).

$$MT = \frac{\sum n.t}{\sum n} \quad \text{(Eq.1)}$$

n = number of newly emerging seedlings/germinated seeds at a time t, t = days from sowing, and Σ n = total emergence seedling/germinated seed.

$$VI = \left(\frac{G1}{D1}\right) + \left(\frac{G2}{D2}\right) + \cdots + \left(\frac{GL}{DL}\right) \quad \text{(Eq.2)}$$

G1 = number of emerged/germinated seeds (first count), D1 = number of days to first count, GL = number of emerged/germinated seeds (last count), and DL = number of days to last count.

Seedling and stem length (cm), stem diameter (mm), leaf length and width (cm), leaf number per seedling, and stem and root fresh and dry weight (g) in randomly selected seedlings that have reached planting size were determined. Stem and root weight loss (g) are calculated from the differences in fresh and dry weights of seedlings.

The study conducted germination and emergence tests using a randomized blocks experimental design with three replications. All data were subjected to analysis of variance (ANOVA), and the mean value was compared with the LSD test. Statistical analyses are conducted using R statistical analysis software version 4.1.0. (Core, 2013) and Agricolae library (de Mendiburu & de Mendiburu, 2019).

RESULTS AND DISCUSSION

According to the results of the germination test, the germination percentage ranged from 100.00% to 86.67% and was statistically insignificant (Table 1). The mean germination time (MTG) among treatments varied between 6.20 and 4.35 days. All *Culex* sp. derived LRWs had a shorter MTG than the control. The 256X treatment of *Culex* sp. had the shortest MTG, while the seeds of CS8 treatment germinated for the longest time. All *Culiseta* sp. treatments had a longer MTG than the control (except for CS3 in the same group as the control). In contrast, most *Culiseta* sp. treatments extended the main germination time, implying slower germination. Yet, all *Culiseta* sp. treatments, except for CS3, were still faster than the control, pointing to their potential utility in enhancing germination efficiency, albeit to a lesser extent than *Culex* sp. derived treatments.

Table 1. The characteristics of LRWs-treated tomato seeds regard germination and emergence.

Treatment	GP	MTG	VIG	EP	MTE	VIE
CS1	100.00	5.96 de	4.58	100.00	13.68	1.51
CS3	97.33	5.28 abcde	5.19	95.00	13.74	1.47
CS4	96.00	5.41 bcde	4.83	100.00	13.62	1.50
CS7	97.33	5.63 cde	4.63	100.00	13.29	1.55
CS8	100.00	6.20 e	4.51	98.33	13.25	1.54
4X	96.00	5.02 abcd	5.32	100.00	13.25	1.59
8X	86.67	4.76 abc	4.80	100.00	13.48	1.51
16X	96.00	5.18 abcd	5.06	100.00	13.85	1.52
32X	98.67	4.83 abc	5.83	100.00	13.71	1.51
64X	96.00	4.62 ab	5.52	95.00	13.77	1.43
128X	98.67	4.82 abc	5.42	98.33	13.58	1.50
256X	93.33	4.35 a	5.56	100.00	13.33	1.55
C	97.33	5.36 abcde	4.88	100.00	13.33	1.53
FFW	96.00	4.89 abc	5.38	96.67	13.23	1.54

GP; Germination Percentage (%), MTG; Mean Time of Germination (days), VIG; Vigor Index of Germination, EP; Emergence Percentage (%), MTE; Mean Time of Emergence (days), VIE; Vigor Index of Emergence

There were no statistically significant differences in the vigor index of germination, emergence percentage, mean time of emergence, or vigor index of emergence. The vigor index of germination ranged from 4.51 (CS8) to 5.83 (32X), and the control was recorded as 4.88. Emergence percentage was generally high and ranged from 100.00 % to 95.00 %; control was recorded as 100.00 %. The mean time of emergence ranged from 13.85 (16X) to 13.23 (FFW) days, and the meantime of all groupswas 13.50 days. The vigor index of emergence ranged from 1.59 (4X) to 1.43 (64X).

The longest plant length was observed in the control group plants (34.74 cm), and the shortest was in the 128X group (28.05 cm). Even though all groups showed less plant length than the control, the *Culiseta* treatment groups were more like the control group. The 256X group that showed the best result respecting the mean germination time (4.35 days) also recorded shorter seedlings (28.59 cm) than the mean of all *Culex*-derived treatment groups (29.86 cm) (Table 2).

The stem length ranged from 10.31 cm to 11.19 cm, the leaf width was 4.41 cm to 6.46 cm, and the leaf length was 4.67 cm to 5.68 cm. There were no statistically significant differences among treatments concerning these three characteristics, but differences in the number of leaves per seedlings were important, and the highest and lowest values were observed in 4X with 3.63 and in CS1 with 2.77, respectively. The stem diameter for the average of all treatments was 2.71 mm and the largest stem diameter was 2.86 mm (128X), the smallest stem diameter was 2.51 mm (4X), and the control group was 2.76 mm (Figure 1).

Although the difference among the treatments is not significant, the recorded highest stem fresh weight was 1.24 g, the lowest was 0.93 g, and the average was 1.09 g. There were statistically significant variations between groups in root fresh weight. The FFW treatment group had the highest RFW (0.81 g), whereas the 64X treatment group had the lowest (0.64 g). Both stem and root dry weight parameters had no statistically significant differences. The stem dry weight varied from 0.18 g to 0.13 g and averaged 0.15 g, whereas the root dry weight ranged from 0.10 g to 0.05 g and averaged 0.06 g (Table 3).

Significant differences were found among seven distinct statistical groups when considering both stem and root weight loss. Regarding stem and root, the 4X treatment group showed the highest weight loss, with values of 1.06 g and 0.75 g, respectively. On the other hand, the 64X treatment group displayed the lowest weight loss in both stem and root, with recorded values of 0.78 g and 0.57 g, respectively. The average stem weight loss was determined to be 0.93 g, and the average root weight loss was 0.64 g. These values were similar to the weight losses observed in both stem and root within the control group (Figure 2).

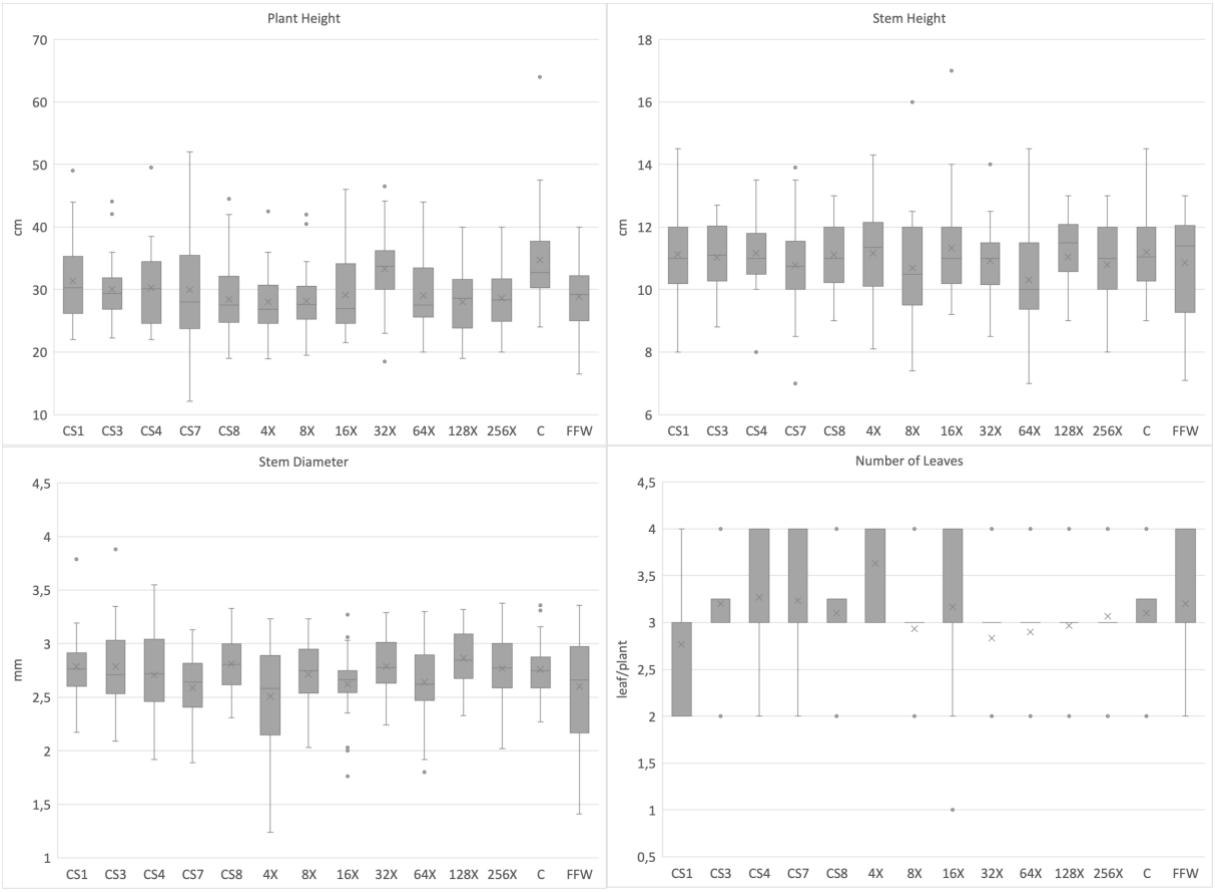

Figure 1. Changing the tomato seedling characteristics according to treatments

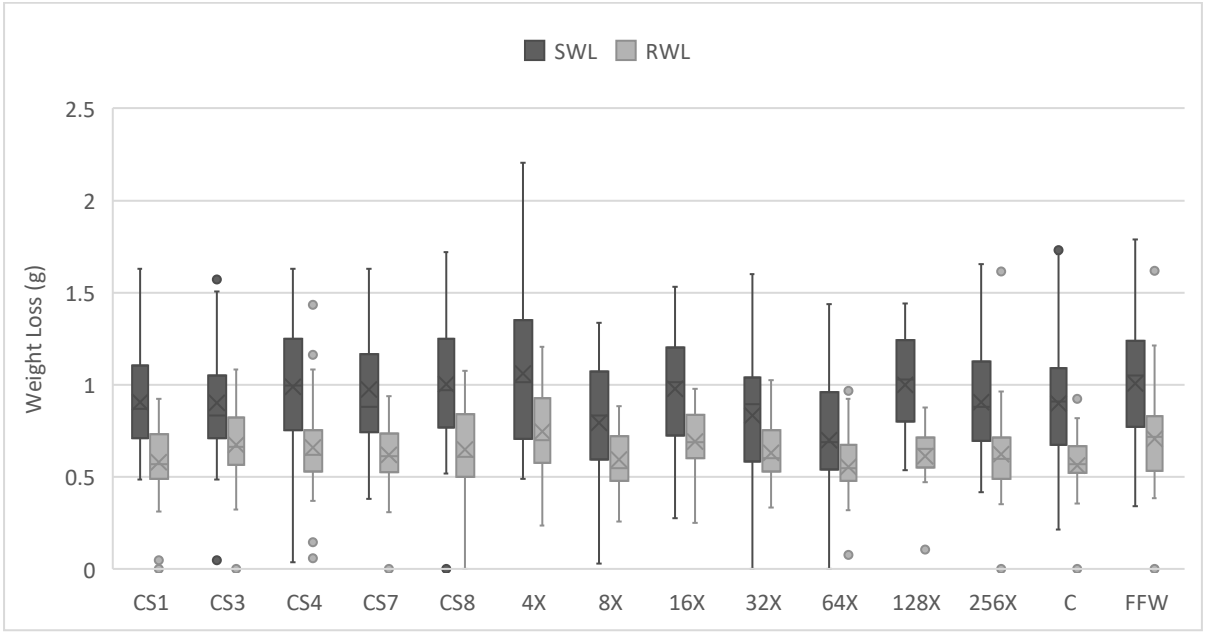

Figure 2. Changing of root and stem weight loss According to different treatments (SWL; stem weight loss, RWL; root weight loss)

Table 2. The effect of different LRWs on some characteristics of tomato seedlings

Treatment	PL	SL	LW	LL	NL	SD
CS1	31.41 abc	11.12	4.88	4.92	2.77 c	2.79 ab
CS3	30.06 abc	11.03	5.01	4.91	3.20 abc	2.79 ab
CS4	30.27 abc	11.16	5.03	5.14	3.27 ab	2.71 abc
CS7	29.98 abc	10.77	4.56	4.69	3.23 ab	2.59 bc
CS8	28.45 c	11.11	4.82	5.41	3.10 bc	2.81 ab
4X	28.05 c	11.16	4.79	4.79	3.63 a	2.51 c
8X	28.21 c	10.70	4.73	5.68	2.93 bc	2.71 abc
16X	29.16 bc	11.34	5.13	4.92	3.17 bc	2.62 abc
32X	33.31 ab	10.93	4.65	4.90	2.83 bc	2.79 ab
64X	29.01 bc	10.31	4.41	4.67	2.90 bc	2.64 abc
128X	28.05 c	11.05	5.06	5.21	2.97 bc	2.86 a
256X	28.59 bc	10.80	4.87	4.80	3.07 bc	2.77 ab
C	34.74 a	11.19	6.46	5.02	3.10 bc	2.76 abc
FFW	28.83 bc	10.86	4.73	5.01	3.20 abc	2.60 bc

PL; Plant Length (cm), SL; Stem Length (cm), LW; Leaf Width (cm), LL; Leaf Length (cm), NL; Number of Leaves, SD; Stem Diameter (mm)

Table 3. The effect of different LRWs on fresh and dry weights of tomato seedlings

Treatment	SFW	RFW	SDW	RDW	SWL	RWL
CS1	1.06	0.65 bc	0.15	0.05	0.91 abcd	0.60 ab
CS3	1.08	0.77 abc	0.18	0.08	0.90 abcd	0.70 bcd
CS4	1.16	0.75 abc	0.17	0.09	0.99 bcd	0.66 abcd
CS7	1.11	0.69 abc	0.13	0.05	0.97 abcd	0.64 abcd
CS8	1.16	0.72 abc	0.15	0.06	1.04 cd	0.67 abcd
4X	1.24	0.80 ab	0.18	0.06	1.06 d	0.75 d
8X	0.93	0.66 abc	0.13	0.06	0.79 ab	0.59 ab
16X	1.13	0.77 abc	0.15	0.08	0.98 bcd	0.69 abcd
32X	1.03	0.68 abc	0.16	0.05	0.86 abc	0.63 abcd
64X	0.96	0.64 c	0.15	0.07	0.78 a	0.57 a
128X	1.19	0.71 abc	0.15	0.10	1.03 cd	0.61 abc
256X	1.05	0.70 abc	0.15	0.06	0.90 abcd	0.65 abcd
C	1.11	0.67 abc	0.17	0.05	0.93 abcd	0.60 ab
FFW	1.14	0.81 a	0.14	0.07	1.01 cd	0.73 cd

SFW; Stem Fresh Weight (g), RFW; Root Fresh Weight (g) SDW; Stem Dry Weight (g), RDW; Root Dry Weight (g), SWL; Stem Weight Loss (g), RWL; Root Weight Loss (g)

Some bio-compounds can improve seedling features such as height, diameter, or weight. For example, a study investigated the effects of *Trichoderma* isolates on tomato seedlings and showed that applications of the isolates significantly increased shoot height and diameter, as shoot and root fresh and dry weight in seedlings (Azarmi et al., 2011). In our study, there were no statistically significant differences in stem length, but LRW and FFW showed shorter seedling lengths than the control (Table 2). The stem diameters of seedlings obtained from priming seeds with two distinct larval-rearing waters with diverse larval densities, and fry food-applied water has considerably varied, and generally, LRW treatments showed more wide stem diameters than the control. According to the plant length, stem length, and stem diameter results, LRW and FFW treatments had a negative effect on root development but good support for thicker plants in tomatoes which are more favourable for seedlings (Azarmi et al., 2011). Considering seedling production, quality, and transportation, thick seedlings that are not tall are generally preferred. In this regard, 128X could be suggested because it was better than others (including control) as a priming treatment.

All bio-compounds don't show similar effects. For instance, irrigation with cyanobacteria-contaminated waters caused biomass loss in tomatoes, especially root biomass (Levizou et al., 2017). Another study found several plant

and microbial metabolites can reduce emergence and negatively influence root and shoot fresh weight in tomato seedlings (Jung et al., 1999). In our research, applications with various sourced LRWs had varying results. *Culiseta* sp. derived LRW resulted in the same or more weight reduction than the control in aspects of the stem and root weight loss. On the other hand, *Culex* sp. 64X treatment gave the best result in these two aspects.

Another study found no variations in germination percentage but substantial differences in germination vigor index when wastewater was reused in broad bean irrigation (Shannag et al., 2021). There were no statistical differences in germination and emergence characteristics, except in the mean germination time. However, there were significant differences in plant length, number of leaves, stem diameter, root fresh weight, stem and root weight loss.

It is possible that the using the wastewater obtained from fish farming in vegetable seedling production. In a study dealing with it's using, it has demostrated that it can be used as irrigation water of *Tabebuia aurea* seedlings by diluting 25-50 % (Pinto et al., 2016). However, it has been seen that the higher concentration results in adverse effects. In our study, there was no linear relationship between concentrations (each concentration has its unique effect)

CONCLUSION

The results of this study suggest that *Culex* and *Culiseta* sp. derived LRWs can significantly influence the mean time of germination of seeds and seedling growth characteristics of tomato seedlings. While the germination percentage was found to be statistically insignificant, the mean germination time varied considerably among the treatments. Interestingly, *Culex* sp. derived LRWs were found to reduce the mean germination time, thus promoting faster germination. This finding is noteworthy as rapid germination is generally associated with improved seedling establishment and could be advantageous in both field and seedling growing conditions. Most other germination and growth characteristics did not vary significantly among treatments, suggesting that while LRW treatments can influence germination time, their impact on other early growth aspects may be limited. However, we also had good results like 128X treatment, which led to shorter but thicker seedlings, which are favourable seedling characteristics.

This study aligns with previous research indicating that LRWs affect seed germination and seedling growth, while demonstrating that effects can vary significantly depending on the LRW source. Importantly, the recycling of biological waste like LRW not only reduces environmental pollution but also transforms waste into valuable agricultural inputs. The recycling of biological waste process is crucial for sustainability, promoting a circular economy, enhancing resource efficiency, and reducing reliance on synthetic chemicals. These advantages of recycling underline the potential of LRW to contribute significantly to sustainable agriculture and improved productivity. Future research should focus on confirming these findings and investigating the underlying mechanisms, particularly the role of different compounds in *Culex* and *Culiseta* sp. derived LRWs and their physiological impacts on seed germination and seedling growth.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflicts of interest.

Author contribution

NS: Methodology, Investigation, Data analysis, Writing-Original Draft, Review & Editing; LA: Supervision, Funding acquisition, Conceptualization, Methodology, Review & Editing; EB: Methodology, Investigation, Data Collecting; EU: Methodology, Investigation, Data Collecting

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Determination of body measurements of Turkish grey cattle with different image processing methods

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Abstract

Twenty heads one-year-old Turkish Grey Cattle Breed, which were protected as part of the conservation of native genetic resources at the Sheep Breeding Research Institute, were evaluated as a material. Body measurements for each animal were determined using the classical method (CM) and six different image processing methods: Fixed Scale Photography (FSP), Fixed Object Photography (FOP), Laser Pointer Photography (LPP), Fixed Scale Video (FSV), Fixed Object Video (FOV), and Laser Pointer Video (LPV), and the methods were compared. The correlation coefficients between CM and FSV, FOV, and LPV were calculated as 0.906 ($p<0.01$), 0.906 ($p<0.01$), and 0.909 ($p<0.01$), respectively for withers height (WH). For back height (BH), the correlation coefficients between CM and the same methods were calculated as 0.879 ($p<0.01$), 0.950 ($p<0.01$), and 0.944 ($p<0.01$), respectively. In terms of rump height (RH), the highest measurement difference was observed between CM and FSV with 3.11%, and the lowest difference was observed between CM and FOV with 0.07%. It was determined that Image Processing Methods (IPMs) could be used as an alternative to classical measurement methods for determining WH, BH, RH, and chest deepth (CD) of each type. It was determined that all IPMs could be used as alternative instead of CM for determining the body measurements of Turkish Grey cattle.

Keywords: Morphometric Measurements, Withers Height, Image Processing, Live Weight, Turkish Grey Cattle

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INTRODUCTION

The physical appearance characteristics of domestic animals have been one of the most discussed topics among animal breeders. The subjects of discussion generally include the measurement tools and methods used to determine these characteristics. Significant differences are observed among animal breeds both within themselves and between each other in terms of physical appearance characteristics. While molecular techniques are used to determine breeds and types in animals, morphological characteristics are generally utilized in current. It has been reported by various researchers that one of the fundamental topics of animal breeding is the evaluation of the physical appearance characteristics of animals (Diekman, 1991, Sekerden and Tapki, 2003).

Classical measurement methods are generally used to obtain data on body characteristics in domestic animals. Measurement tools used in classical methods include the measuring stick (Lydin's Stick), measuring compass (Duerst's goniometer), and measuring tape (Nilipour and Butcher, 1997). Many researchers have noted that measuring large animals such as cattle and buffalo, small-sized animals like goats and lambs, and wild and semi-domestic animals using classical methods can be time-consuming, difficult to apply, and prone to errors. The results obtained from studies conducted by different researchers for morphological evaluation can be contentious (Zehender et al., 1996). During the development of computer-based technologies, many processes that were difficult and time-consuming until recently have become simpler and less time-consuming. Among these technological applications, 'Image Processing Methods', which have origins in space research, have started to be

used in the field of animal husbandry in recent years, as in many other fields (Grashorn and Komender, 1991; Aktan, 2004).

The applicability of different IPMs in determining body measurements of Turkish Grey cattle, which are protected within the scope of the conservation of native genetic resources, was evaluated. For this purpose, body measurements of the breed were determined using different Image Processing Methods (IPM) and Classical Methods (CM), and the methods were compared in this study.

MATERIALS AND METHODS

Material

The animal material for the research consisted of twenty head of one year old Turkish Grey Cattle Breed raised at the Sheep Breeding Research Institute, affiliated with the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM). These animals were bred for the conservation of genetic resources. The body measurement points for Turkish Grey Cattle Breed were determined based on the measurement points reported by Ilaslan et al., (1983) for cattle and buffaloes.

a. Withers Height (WH): The length of the vertical line from the highest point of the withers (spinous process of the 4th thoracic vertebra) to the ground.

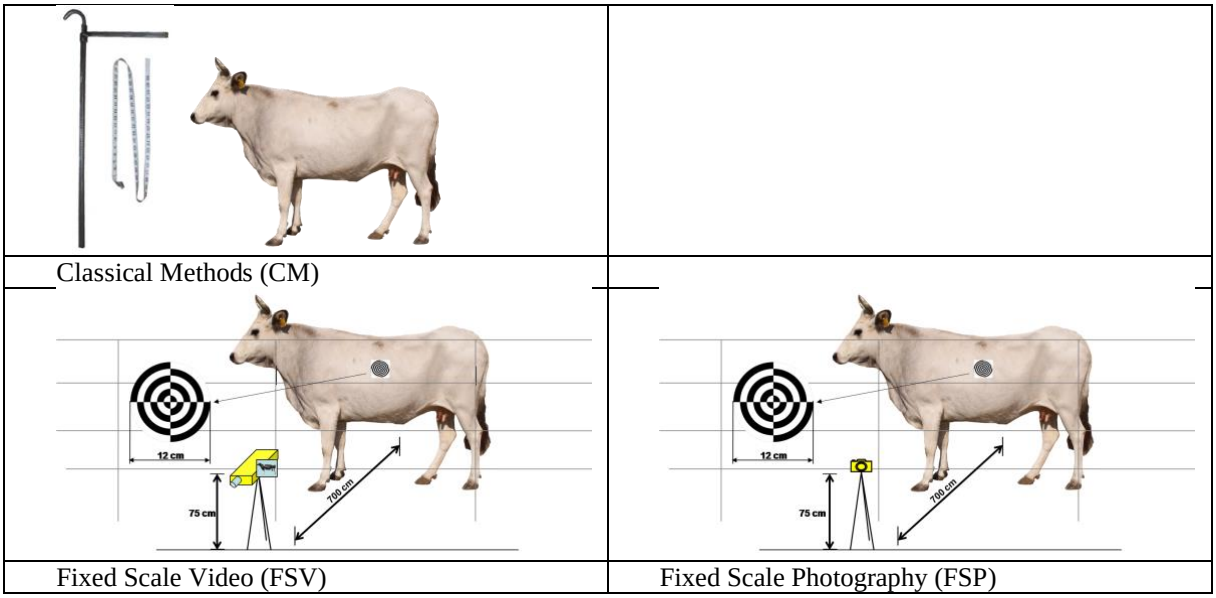
b. Back Height (BH): The distance from the spinous process of the last thoracic vertebra (13th thoracic vertebra) to the ground.

c. Rump Height (RH): The height from the highest point of the sacrum (most dorsal part of the sacrum at the level of the tuber coxae) to the ground.

d. Chest Depth (CD): The depth from the highest point of the withers (spinous process of the 4th thoracic vertebra) to the sternum (ventral surface of the sternum).

Image processing methods (IPMs)

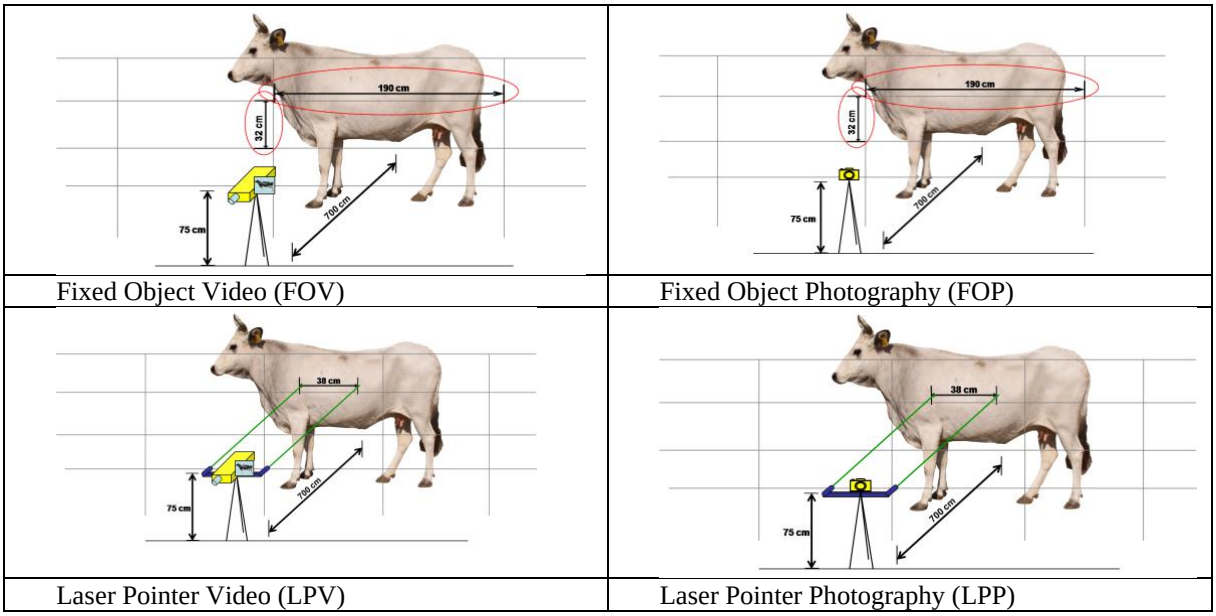
In the method described as classical or traditional, body measurements were taken using measuring sticks and measuring tapes known as measurement tools (Nilipour and Butcher, 1997). In the Fixed Scale Video (FSV), Fixed Object Video (FOV), and Laser Pointer Video (LPV) methods, video images were captured using a Sony HDR-CX105E® camera, while the Fixed Scale Photography (FSP), Fixed Object Photography (FOP), and Laser Pointer Photography (LPP) methods utilized a Canon Digital IXUS-900TI® camera. The image capture devices were fixed on tripods at a height of 75 cm. All images were recorded from a distance of 700 cm. For the FSV and FSP methods, a fixed circular scale with a diameter of 12 cm, divided into segments and colored at one-centimeter intervals, was placed on the animals as a reference source. In the FOV and FOP methods, a fixed object was used as the reference source. For this purpose, two different reference points were identified on a platform created for the animals' passage (190 cm and 32 cm). In the LPV and LPP methods, laser pointers emitting green light with wavelengths and powers (532 nm wavelength and 5mW power) compliant with International Animal Welfare criteria (ANSI 2000; IEC 1998) were used as the reference source. These details provide clarity on the equipment and methods used for capturing images and establishing reference points in the study (Figure 1).



Classical Methods (CM)

Fixed Scale Video (FSV)

Fixed Scale Photography (FSP)



Fixed Object Video (FOV) | Fixed Object Photography (FOP)

Laser Pointer Video (LPV) | Laser Pointer Photography (LPP)

Figure 1. Image processing methods

Image processing stages

For processing and analyzing digital images obtained through Image Processing Methods, Image Pro-Plus 4.5 Demo © software developed by Media WHbernetics, Inc. (MD-USA, 1995-2001) was utilized (Figure 2). In the study, data obtained from classical measurements and image processing methods were compared statistically.

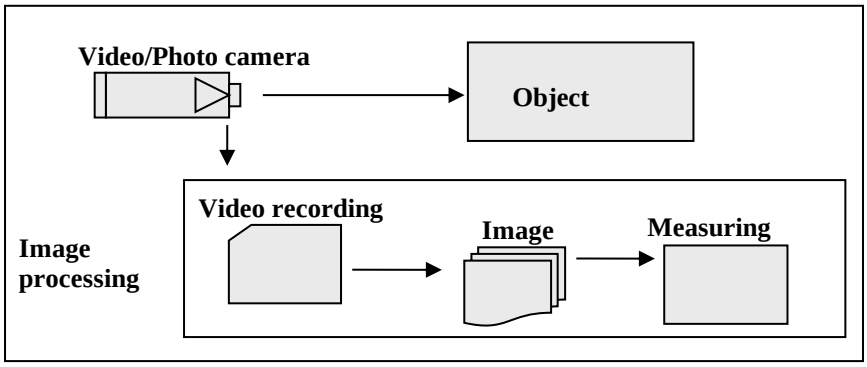


Figure 2. Image processing stages

Variance Analysis Method (ANOVA) was used to determine the difference between the average of various body measurements using CM and IPMs. The Duncan Multiple Range Test was used to determine which group means are significantly different (Düzgüneş ve ark. 1993). Linear Regression model was used for determination of regression equations. The SPSS statistical software (1993) was used for the analysis and evaluation of the results.

RESULTS AND DISCUSSION

The body measurements obtained using both methods in Turkish Grey cattle are presented. It was observed that WH measured with CM was 97.05 cm, whereas measurements obtained with IPM ranged from 92.87 cm (FSV) to 98.08 cm (FSP). Regarding WH, the highest difference was observed between CM and FSV at 4.31%, while the lowest difference was 0.02% with FOV. Furthermore, positive and significant correlations were found between CM and other methods. Specifically, the correlation coefficients between CM and FSV, FOV, and LPV were calculated as 0.906 ($p<0.01$) each, while for FSP, FOP, and LPP they were 0.896 ($p<0.01$), 0.871 ($p<0.01$), and 0.896 ($p<0.01$) respectively (Table 1).

In studies related to WH height, previous research (Kuchida et al., 1995; Zehender et al., 1996; Ozkaya, 2006; Tozsér et al., 2000; Polak et al., 2007; Ozder and Onal, 2008) on various species such as cattle, calves, bulls, and dairy cows have reported high compatibility between IPM and CM in determining WH, with correlation coefficients ranging from 0.391 to 0.98, and significant levels ($P<0.05$ or $P<0.01$). The findings of our study align with these literature results, demonstrating generally high compatibility between IPM and CM methods. In the study of Turkish Grey cattle, Table 1 presents the body measurements obtained using both the CM and IPM

methods for the variable BH. CM measured BH as 97.90 cm, whereas measurements obtained with IPM ranged from 94.06 cm (FSV) to 98.27 cm (FSP). In terms of BH, the highest difference was observed between CM and FSV at 3.92%, while the lowest difference was 0.05% with LPV.

Table 1. Body measurement

	Methods	$\overline{X}$ ±SE	VC	r	B	b	R^2 (%)	e	Diff. (%)
		n=20							
	CM	97,05±1,35	6,24						
	FSV	92,87±1,21	5,82	0,906**	2,7	1,02	82,1	6,92	4,31
	FOV	97,07±1,21	5,60	0,906**	-1,0	1,01	82,1	6,94	0,02
WH	LPV	97,52±1,27	5,80	0,909**	2,2	0,97	82,7	6,70	0,48
	FSP	98,08±1,34	6,10	0,896**	8,0	0,91	80,3	7,64	1,06
	FOP	95,94±1,30	6,07	0,871**	10,1	0,91	75,9	9,33	1,14
	LPP	95,07±1,24	5,86	0,896**	4,3	0,98	80,3	7,62	2,04
	Total	96,23±0,49	6,05						
	F	1,960							
		n=20							
	CM	97,90±1,43	6,53						
	FSV	94,06±1,31	6,23	0,879**	7,70	0,96	77,3	9,79	3,92
	FOV	96,10±1,40	6,52	0,950**	4,87	0,97	90,2	4,21	1,84
BH	LPV	97,95±1,56	7,10	0,944**	13,0	0,87	89,0	4,73	0,05
	FSP	98,27±1,44	6,55	0,911**	9,07	0,90	83,0	7,34	0,38
	FOP	95,92±1,43	6,66	0,910**	10,6	0,91	82,8	7,39	2,02
	LPP	95,17±1,51	7,08	0,933**	13,6	0,89	87,1	5,56	2,79
	Total	96,48±0,55	6,71						
	F	1,239							
		n=20							
	CM	100,65±1,41	6,29						
	FSV	97,52±1,38	6,32	0,908**	9,75	0,93	82,4	7,46	3,11
	FOV	100,72±1,54	6,85	0,953**	12,6	0,87	90,8	3,91	0,07
RH	LPV	102,32±1,67	7,30	0,928**	20,3	0,79	86,0	5,90	1,66
	FSP	102,66±1,68	7,32	0,926**	20,6	0,78	85,8	6,00	2,00
	FOP	100,11±1,62	7,25	0,920**	20,4	0,80	84,6	6,51	0,54
	LPP	99,22±1,64	7,38	0,918**	21,9	0,79	84,3	6,64	1,42
	Total	100,46±0,60	7,02						
	F	1,265							
		n=20							
	CM	42,95±0,81 b	8,43						
	FSV	44,90±0,58 b	5,79	0,683**	0,3	0,95	46,7	7,37	4,54
	FOV	47,21±0,70 a	6,60	0,806**	-1,24	0,94	65,0	4,84	9,92
CD	LPV	48,25±0,74 a	6,87	0,576**	12,6	0,63	33,2	9,24	12,34
	FSP	48,55±0,80 a	7,35	0,768**	5,11	0,78	59,0	5,67	13,04
	FOP	47,66±0,89 a	8,39	0,658**	14,6	0,60	43,4	7,83	10,97
	LPP	47,76±0,70 a	6,53	0,714**	3,35	0,83	51,0	6,77	11,20
	Total	46,75±0,32	8,13						
	F	7,500**							

a-b: Means within rows with different superscript alphabets are significantly different (**; p<0.01). (VC: Coefficient of variation, r: correlation coefficient, R^2: The coefficient of determination, e:error)

Positive and significant correlations were found between CM and other methods. Specifically, the correlation coefficients between CM and FSV, FOV, and LPV were calculated as 0.879 (p<0.01), 0.950 (p<0.01), and 0.944 (p<0.01), respectively. For FSP, FOP, and LPP, the coefficients were 0.911 (p<0.01), 0.910 (p<0.01), and 0.933 (p<0.01), respectively. In studies related to BH measurement, previous researchers (Zehender et al., 1996; Ozkaya, 2006; Ozder and Onal, 2008) have reported high compatibility between IPM and CM methods in various species, including cattle and sheep, with correlation coefficients ranging from 0.86 to 0.93, and significant levels (P<0.01).

These studies have demonstrated that there is generally no statistically significant difference between CM and IPM methods in determining BH.

In the study of Turkish Grey cattle, the average value of RH measured by CM was 100.65 cm, while measurements obtained with IPM ranged from 97.52 cm (FSV) to 102.72 cm (SFOV). Regarding RH, the highest difference was observed between CM and FSV at 3.11%, while the lowest difference was 0.07% with FOV. Positive and significant correlations were found between CM and other methods. Specifically, the correlation coefficients between CM and FSV, FOV, and LPV were calculated as 0.908 ($p<0.01$), 0.953 ($p<0.01$), and 0.928 ($p<0.01$), respectively. For FSP, FOP, and LPP, the coefficients were 0.926 ($p<0.01$), 0.920 ($p<0.01$), and 0.918 ($p<0.01$), respectively. In previous studies, Bianconi and Negretti (1999) reported a correlation coefficient of 0.96 for cattle, Polak et al. (2007) found a coefficient of 0.66 for bulls using IPM, Core et al. (2008) reported coefficients ranging from 0.66 to 0.74 for cattle, and Ozder and Onal (2008) found a coefficient of 0.91 for cattle. In studies involving different species, Negretti et al. (2004) reported a coefficient of 0.97 for goats ($P<0.01$), and Onal and Ozder (2008) reported a coefficient of 0.83 for sheep, indicating high compatibility between IPM and CM in determining RH. However, it is worth noting that Kuchida et al. (1995) reported a lower correlation coefficient of 0.198 ($P>0.05$) for cattle using IPM compared to other studies. This suggests variability in the reported correlations across different research efforts.

The average value of CD measured by CM was 42.95 cm, while measurements obtained with IPM ranged from 44.99 cm (FSV) to 48.55 cm (FSP). Regarding CD, the highest difference was observed between CM and FSP at 13.04%, while the lowest difference was 4.54% with FSV for Turkish Grey Cattles. Positive and significant correlations were found between CM and other methods. Specifically, the correlation coefficients between CM and FSV, FOV, and LPV were calculated as 0.683 ($p<0.01$), 0.806 ($p<0.01$), and 0.576 ($p<0.01$), respectively. For FSP, FOP, and LPP, the coefficients were 0.768 ($p<0.01$), 0.658 ($p<0.01$), and 0.714 ($p<0.01$), respectively. In previous studies, Kuchida et al. (1995) reported a correlation coefficient of 0.65 for cattle, Bianconi and Negretti (1999) found a coefficient of 0.86 for cattle, Tozsér et al. (2000) reported coefficients of 0.86 for calves ($P<0.01$) and 0.87 for dairy cattle ($p<0.01$), Ozkaya (2006) reported a coefficient of 0.94 for cattle ($p<0.01$), and Ozder and Onal (2008) found a coefficient of 0.87 for cattle ($p<0.01$). In studies involving different species, Onal and Ozder (2008) reported a coefficient of 0.77 for sheep ($p<0.01$). These studies indicate high compatibility between IPM and CM in determining CD. However, Maroti-Agóts et al. (2005) reported a statistically significant difference between IPM and CM in determining CD ($P<0.01$). The higher proportional difference between the two methods in CD could be attributed to variations in the animals' back and abdomen hair condition.

CONCLUSION

In Turkish Grey Catte, it has been determined that Image Processing Methods can be used to measure body dimensions such as WH, BH, RH, and CD. Overall, it has been found that the relationship between the two methods is high and statistically significant across all measurement points. The lower correlation observed in CD between the two methods may be attributed to the animals' back and abdominal wool condition. Methods utilizing video cameras for image capture provide the capability to capture animal images at desired points, which can potentially yield more accurate results.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

ARÖ: Designed, and performed the experiment; analyzed the data; wrote the paper. **MÖ:** Designed and performed the experiment analyzed the data, and reviewed the article. All authors read and approved the manuscript.

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Ethics committee approval

The experiment was approved as no needed ethics approval by the Scientific Committee Department of Animal Science at the Namik Kemal University. Cert. no. of use of lab. Anim./IACUC number: 2837713 AALAS Learning Lab. 10/1/2013

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Olive oil consumption, preferences and usage areas: the case of Hatay and Aydın provinces

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Abstract

Olive and olive oil are essential food sources for their health benefits and flavour. It is crucial in regions such as Aydın and Hatay, where olives are intensively grown. This research was conducted through focus group discussions, in-depth interviews, and a questionnaire survey to understand how olive oil is preferred in cooking and its different uses in health, carpentry, skincare, hair care, massage oil, and aromatherapy. The study's findings show that the versatile use of olive oil is widespread in Aydın and Hatay provinces. The results of this research can be used for olive oil producers and marketers to develop sales and marketing strategies and better understand the potential uses of olive oil. Local production and price were the determinants of consumers' olive oil preferences. The results of this study reveal the diversity of olive and olive oil use in Aydın and Hatay provinces and how consumers prefer the product in different areas. Within the scope of the research, a questionnaire survey was conducted in two regions, and a scale of 1-5 points was used to help the respondents give more precise answers to the statements in the form. The findings were analysed using ordinal ranking and one-factor multiple variance analysis MANOVA. The results of this research were used to develop sales and marketing strategies for olive oil producers, marketers and consumers and to understand the potential uses of olive oil better.

Keywords: Olive oil, Areas of use, Consumer preferences, Olive Oil Quality, Price

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INTRODUCTION

Olive oil is a highly pure, clear and healthy source of vegetable oil with a greenish-yellow colour and a distinctive odour, extracted by different methods from the nuts of the Olea Europa plant (Wikipedia, 2024). Olive oil, which is the only oil that physical methods can produce, has always had a higher economic value compared to other vegetable oils because it requires much manual labour in the collection phase of its raw material (olives) and requires significant technology compared to other seed oils in the stage of obtaining from the grain, mainly because it is a beneficial food source in the field of health.

Archaeological studies and research have shown that olive oil was used in ancient times to prevent foot injuries during long journeys, to soften the body in physical sports, in oil lamp lighting by taking advantage of the flammable properties of the oil, in medicine making, nutrition and cosmetic purposes (Kaplan and Arıhan, 2012, p. 8). Olive cultivation and consumption are important in Turkey, especially in the Aegean and Mediterranean regions (Sakar and Ünver, 2011, pp. 19-25). Hatay and Aydın provinces are among the prominent regions in this field. The privileged climate and soil structure of Hatay and the fertile soils of Aydın enable the cultivation of quality olives and the production of delicious olive oils. When the consumption of olive oil is analysed, it has increased in the last decade, and its importance in the global sector has also increased (Özkan Z., 2021, pp. 4).

According to the data obtained from the Provincial Directorates of Aydın Ministry of Agriculture and Forestry, Memecik, Domat, Gemlik, Manzanilla, Donkey Olive, Yamalak Yellow, Arbequina (Spanish origin, early fruiting

dwarf olive) are the most common olive varieties in Aydın province (Zeytin Kitabı, 2019:13). In Hatay province, Halhali olive, Saurani (Sinonimi Savrani), Attun (Black olive), Köstüklü (Kilyeli green olive), Sarı Habeşi, Gemlik olive, Har olive, Büyük Topak Ulak, Kilis Oil and Nizip oil are the various types of olives grown in this region due to the neighbouring lands (Konuşkan and Canbaş, 2008, pp. 58). The crops from the trees growing in both regions and the oil obtained from the crop are divided into different varieties; each oil has different uses. Especially in the in-depth interviews conducted with the qualitative research method used in this study, it was determined that olive oil obtained from the trees is widely used by the region's people, especially in cooking, skin, hair, cleaning, cosmetics and especially in the health field.

Hatay olives and olive oil have a more intense and fruity flavour, while Aydın olives and olive oil have a milder and more balanced aroma. Aydın olive oil is mainly preferred for salads and cold dishes, while Hatay olive oil is used for hot and meaty dishes. For example, Aydin Olive Oil is used in Artichokes, Zucchini with Olive Oil, Beans with Olive Oil, Shepherd's Salad and Olive leaf wraps, while Hatay olive oil is preferred in dishes such as Ali Nazik, Vicious, Tray Kebab, Içli köfte, Frying and Simit Kebab. Halhali and Saurani olives stand out in Hatay and Memecik olives stand out in Aydın (Oğuzhan M. U, 2022). The differences in the areas of use of the two regions are due to the variability of the climate, soil and production methods used in the regions where olives and olive oil are grown. Due to these differences, Hatay olive oil contains more spicy and intense flavors, while Aydın olive oil contains a lighter and fruity taste, and olive oil used in the same recipes may have different results. Both provinces have olive and olive oil cultures, contributing significantly to culinary habits and local economies.

MATERIALS AND METHODS

This research was designed as an applied study and carried out with a descriptive approach. The main problem of the research is to determine the areas of use of olive oil and its varieties in two important olive growing centers and determining the level of participation in the statements developed about olive oil. Data were obtained using a questionnaire.

Before starting the survey, focus groups and in-depth interviews were conducted using the qualitative method. This method was preferred to understand better olive and olive oil consumption and usage, as well as consumers' experiences, preferences, habits, and emotions. The research was conducted on consumers residing in Aydın and Hatay provinces who are ordinary product users. The study was conducted on olive oil users in these two provinces. The central mass of the research consists of olive oil consumers in these two provinces. In the focus group and in-depth interviews, data were obtained by reaching people who know the subject well by considering the snowball method in the olive and olive oil fields. This study conducted face-to-face interviews with 40 experts, and a focus group study was conducted once with eight people. The questionnaires created were based on the findings obtained from these interviews, which were conducted face-to-face and online. The data obtained through questionnaires were collected in the SPSS 27 program, and Single Factor Multiple Analysis of Variance (MANOVA) was applied. MANOVA (Multivariate Analysis of Variance) is a statistical technique used to analyze the effects of multiple dependent variables together with one or more independent variables.MANOVA allows to evaluate the relationships between dependent variables and group differences Decently at the same time. MANOVA Analysis was used to measure whether there is a difference between the answers given to the statements regarding consumer preferences and usage areas in both provinces (Çakmak, A. Ç., (2012).

PREPARATION OF THE QUESTIONNAIRE

The scale used in this study is based on the findings obtained from focus groups and in-depth interviews conducted using qualitative methods. In addition, a questionnaire form was created by taking into account previous studies (Cömert et al., 2012; Çelik et al., 2016; Güdek et al., 2016; Yaylacı, 2022). In this context, scale development is frequently encountered in studies on the purchasing behaviour of consumers in the literature. However, there is no scale study on the use of crops and oil in different areas. For this reason, the questionnaire was enriched with data obtained through qualitative methods (focus groups and in-depth interviews). The first part of the questionnaire included questions on demographic characteristics. In contrast, the next part included statements from literature studies and questions determining the shopping information, attitudes, behaviours and usage areas of Olive and Olive Oil users. In addition, the respondents' sources of information about olive oil were also asked in the survey.

In the questionnaire form, statements aiming to determine the participants' views are asked using a 5-point Likert scale. The Likert scale offers a five-point evaluation system including "Strongly Disagree" (1), "Disagree" (2), "Neutral" (3), "Agree" (4) and "Strongly Agree" (5) and "Never Use" (1), "Never Use" (2), "Neutral" (3), "Use" (4) and "Always Use" (5). The 1-5 point scale has been used in some studies to measure items developed to question recreation barriers in the village (Ardahan and Öz, 2019, p. 144). This type of scale offers a transparent scoring system to the survey participants, and this scale will allow many averages to be taken and various comments to be made on this study. Moreover, simultaneously, participants can score more objectively than the Likert scale (Çakmak, 2012, pp. 202-203). In addition, data were collected by including open-ended sections in both demographic statements and other question statements.

LITERATURE REVIEW

In a study examining olive oil consumption and behaviours, it was concluded that the widespread production of olives, income, living standards and, most importantly, local food culture, habits and similar factors impact olive oil consumption (Ağır et al, 2018).

A study examining the reasons for olive oil consumers' preference concluded that factors such as quality perception arising from product diversity, price realities, reliability in the production process, taste and flavour, and oil appearance affect purchasing behaviours (Yaylacı, 2022).

In a study examining the areas of use of olive oil, it was concluded that olive oil has many benefits in diseases such as cardiovascular diseases, hypertension, diabetes, hyperlipidemia and cancer and that olive oil has an area of use in the field of health (Armutcu et al., 2013).

A study examining olive oil consumer purchasing behaviours concluded that, after all evaluation studies, efficiency values, and the location of olive pressing facilities are related, as are environmental and quality criteria that affect purchasing behaviours (Kır, 2023).

In a study examining olive oil consumer shopping preferences, a survey was conducted in four critical countries, and the type of olive oil was ranked as the most essential product feature. Moreover, a comparative study determined that olive oil price was the second most important criterion among consumers. It was concluded that country of origin, packaging design, label and brand criteria were among the less important factors influencing purchasing behaviour (Chrysochou et al., 2022).

In a study examining the shopping preferences of consumers for olive oil, it was observed that high-income consumers consider the brand as well-known and reliable. At the same time, they are willing to pay more money and prefer glass bottles in olive oil packaging. At the same time, it was concluded that consumers prioritise health in oil selection, they provide price stability by purchasing more olive oil, the preferred places for olive oil purchase are generally producers or self-production places, not markets, and these factors affect shopping centre preferences (Erbaş & Artukoğlu,2016).

In a study examining the uses of olives and olive oil, various scientific studies supported the health benefits of olive leaf, including antioxidant, antihypertensive, anti-inflammatory, antithrombotic, and cardioprotective effects. It was concluded that more research is needed on these effects, interactions of these substances with other nutrients, optimal dose determination and safety, but the olive leaf has obtained very positive results in the field of cancer, and its use in the field of health may be widespread (Gürbüz and Öğüt (2017).

In a research study on the use of olive and olive oil, it was concluded that olive leaves have immune system strengthening effects and are very rich in phenolic compounds, have many therapeutic effects thanks to the phytochemicals in their composition and have potential for use in food applications (Salık and Çakmakçı (2021).

In a study examining the field of use of olive oil, By emphasising the importance of the Mediterranean diet, it was concluded that olive oil consumption, olive oil consumption, which naturally contains foods rich in fatty acids and is suitable for nutritional recommendations regarding micro and macronutrient composition, among others, antioxidant, immunomodulatory and inflammatory response regulator results in beneficial effects in many different pathologies (Luna et al. (2022).

When the general studies on olive oil consumption are examined, demographic characteristics and factors affecting purchasing behaviours are examined. However, there are limited studies on different uses of olive oil. In this study, various uses of olive oil in two provinces and consumer preferences according to demographic characteristics were examined in detail.

RESEARCH HYPOTHESES

H1: There is a significant difference between consumers in Hatay and Aydın regarding their agreement with the statement that olive oil prices are affordable.

H2: There is a significant difference between consumers in Hatay and Aydın in terms of the level of agreement with the statement that olive oil deserves the money paid.

H3: There is a significant difference between consumers in Hatay and Aydın in terms of level of agreement with the statement that expensive olive oil is of better quality.

H4: There is a significant difference between consumers in Hatay and Aydın in terms of their agreement with the statement that perfect olive oil will burn the throat when drunk.

H5: There is a significant difference between consumers in Hatay and Aydın in terms of their agreement with the statement that packaging is essential when buying olive oil.

H6: There is a significant difference between consumers in Hatay and Aydın in terms of their agreement with the statement that extra virgin olive oil has the highest nutritional value.

H7: There is a significant difference between consumers in Hatay and Aydın in terms of the level of agreement with the statement of trusting branded olive oil more.

H8: There is a significant difference between consumers in Hatay and Aydın regarding their agreement with the statement that buying quality products from familiar producers is necessary.

H9: There is a significant difference between consumers in Hatay and Aydın in terms of level of agreement with the statement that olive oil nourishes the skin, strengthens the nails and nourishes the hair.

H10: There is a significant difference between consumers in Hatay and Aydın in terms of their agreement with the statement that mosquitoes will not bite the skin smeared with olive oil.

H11: There is a significant difference between consumers in Hatay and Aydın in terms of their level of agreement with the statement that olive oil is good for chronic constipation and has a stomach-protective effect.

H12: There is a significant difference between consumers in Hatay and Aydın in terms of level of agreement with the statement that olive oil regenerates cells and protects against cancer.

H13: There is a significant difference between the consumers in Hatay and Aydın regarding the level of participation in the statement that meals made with olive oil are more delicious than those made with other oils.

AN APPLICATION IN HATAY AND AYDIN PROVINCES

Subject of The Research

The research focuses on analysing and determining the areas where olive and olive oil consumers in two provinces use this crop. Olive and olive oil consumers living in Aydın and Hatay, the favourite provinces for olive and olive oil consumption, are included in the subject of this study.

Purpose and Importance of The Research

Olives and olive oil have not only economic but also cultural value. Widely used in Turkish cuisine and traditional medicine (Kaplan & Arıhan, 2012), olives and olive oil are the product of knowledge and experience passed down through many generations. Therefore, an in-depth study of olive and olive oil culture and its adaptation to progressive practices offers essential research and development opportunities for academic and industrial applications. Aydın province, one of Turkey's major olive and olive oil-producing provinces, had 22.662.164 million fruit-bearing olive trees in 2022-2023, yielding an average of 61.609 tons of olive oil (National Olive Report, 2019). Hatay province, on the other hand, according to this report, had 14.130.053 million fruit-bearing trees and 145.000 tons of olives in 2022-2023, with an average olive oil production of 27.000 tons (National Olive Report, 2019). These data show how important the two provinces are in production and consumption. This study aims to understand these regions' olive oil consumption patterns by examining olive oil consumption and preferences in two provinces that are essential production centres of olives and olive oil. The findings and inferences from the different uses of olives and olive oil provide critical perspectives to the stakeholders in the olive oil market and the industry. The central mass of the research consists of olive oil consumers in Hatay and Aydın provinces. This study aims to provide valuable information to stakeholders in the industry and market by measuring producer and consumer consumption and uses of olive oil.

Table 1. Official Data of the Ministry of Agriculture and Forestry

HATAY REKOLTE FORECAST								
YEAR	Number of trees		Olive grain per tree (Kg)	Olives to be obtained (Ton)	Olives to be divided into dishes (Ton)	Olives to be divided into oil (Tons)	Olive oil to be obtained (Ton)	Olive grains for 1 kg of olive oil (Kg)
	Bearing Fruit	Not Bearing Fruit						
2012-2013	11.002.427	3.535.584	9,1	99.682	29.905	69.777	17.444	4,0
2013-2014	11.888.323	2.651.688	8	95.107	23.892	71.215	17.804	4,0
2014-2015	12.418.660	2.121.351	10	124.187	37.256	86.931	21.733	4,0
2015-2016	12.418.660	2.121.351	13,6	168.750	33.750	135.000	27.000	5,0
2016-2017	9.668.778	3.577.115	7	66.180	9.930	56.250	12.500	4,5
2017-2018	12.098.596	3.189.532	11,8	143.000	7.150	135.850	26.000	5,2
2018-2019	12.492.281	3.294.750	7,3	91.000	5.500	85.500	17.000	5,0
2019-2020	12.492.281	3.294.750	9,8	127.500	15.000	112.500	25.000	4,5
2020-2021	13.442.136	2.999.011	5,0	67.211	6.721	60.490	13.442	4,5
2021-2022	13.732.722	3.059.735	8,7	120.000	12.000	108.000	24.000	4,5
2022-2023	14.130.053	2.673.400	10,3	145.000	10.000	135.000	27.000	5,0

Table 2. Official Data of the Ministry of Agriculture and Forestry

AYDIN REKOLTE FORECAST								
YEAR	Number of trees		Olive grain per tree (Kg)	Olives to be obtained (Ton)	Olives to be divided into dishes (Ton)	Olives to be divided into oil (Tons)	Olive oil to be obtained (Ton)	Olive grains for 1 kg of olive oil (Kg)
	Bearing Fruit	Not Bearing Fruit						
2012-2013	21.940.698	2.495.217	7,9	173.639	49.778	123.861	23.830	5,2
2013-2014	22.058.345	5.871.777	5,5	120.421	26.308	94.113	17.343	5,0
2014-2015	22.076.091	2.251.196	7	152.651	48.053	104.598	20.512	5,1
2015-2016	21.941.499	2.408.727	5,3	117.173	39.568	77.605	15.694	4,9
2016-2017	22.201.194	2.324.978	11	283.151	58.239	224.912	37.554	6,0
2017-2018	22.811.443	2.292.496	19,3	440.796	77.676	363.120	68.650	5,3
2018-2019	22.387.754	2.514.812	9,0	200.889	31.985	168.904	33.781	5,0
2019-2020	22.193.288	2.430.472	17,4	334.030	77.049	256.981	52.272	4,9
2020-2021	22.092.794	2.468.514	5,5	120.704	31.622	89.082	16.197	5,5
2021-2022	21.559.929	2.228.534	13,3	287.749	43.693	244.056	42.370	5,8
2022-2023	22.662.164	2.421.767	19,0	429.860	77.906	351.954	61.609	5,7

ANALYSIS AND EVALUATION OF THE DATA OBTAINED FROM THE RESEARCH

Demographic Characteristics and Shopping Information of Surveyed Visitors

Four hundred eight respondents from Hatay and Aydın provinces, especially female olives and olive oil consumers, participated in this study. May July 1, 2024, the survey was conducted in the districts of Hatay and Aydın province between the dates of May 1 and July 1, 2024. The survey was conducted in the districts of Hatay and Aydın Dec. The demographic characteristics of the respondents in this study are given in Table 3. When we look at the demographic characteristics of the respondents who participated in the survey, we see that in Hatay, the interest and interest of young age groups in olive and olive oil consumption is at the upper limit, while in Aydın, the interest of adults between the ages of 25-44 towards these products is higher. These demographic differences may shape olive and olive oil consumer preferences, marketing strategies, and sales tactics in both regions. Again, when the education levels in these two provinces are analysed, most consumers are at the university level. The high education level in Hatay indicates an increase in olive and olive oil consumption, while in Aydın, a more comprehensive range of education levels indicates that these products are widely consumed. When the marital status of the individuals who participated in the survey was analysed, the majority of the respondents in Hatay were single consumers, 52%. In comparison, 60.6% of the respondents in Aydin were married. Consumers who participated in the survey were asked open-ended questions about their income status, and the grouping system in the table was created within the framework of an inevitable comparison. In the group of those who did not specify their income, it was thought that students and homemakers could be included in this group since the respondents gave the answers of students and homemakers in response to the survey statements. The 19,999 TL and below group represents students and low-income respondents earning minimum wage. The group between 20-39,999 TL represents the survey participants in the middle-upper class. The group of 40.000 TL and above is characterised as upper class. While the average income of the survey participants in Hatay is 22672.57, the average income in Aydin is 23388.94. Based on these data, it would be more accurate for olive and olive oil sellers to determine their price levels according to the averages of these two provinces. The olive and olive oil consumers who participated in the study were asked about their occupations in an open-ended question; the participants' occupational information is given in Table 1. Looking at the occupational information of the participants, it is seen that the rate of public employees in Hatay (37%) is higher than the rate in Aydın (25%). On the other hand, the proportion of homemakers in Aydın (36.1%) is higher than in Hatay (24.5%), suggesting that homemakers have a significant share in olive and olive oil consumption in Aydın. While private sector employees have similar shares in both provinces (Hatay 20%, Aydın 22.6%), the number of students is higher in Hatay (16.5%) than in Aydın (10%). The self-employed are overrepresented in Aydın (6.3%) compared to Hatay (2%). These data reveal that olive and olive oil consumer profiles in Hatay and Aydın differ according to occupational groups. Marketing strategies should be shaped according to these differences in occupational groups in both provinces. For example, while campaigns targeting homemakers may be more prevalent in Aydın, strategies targeting public employees may be prioritised in Hatay.

Table 3. Demographic Characteristics of Participants

	HATAY		AYDIN			HATAY		AYDIN	
Age	**F**	**(%)**	**F**	**(%)**	**Education Level**	**F**	**(%)**	**F**	**(%)**
24 years and younger	87	36,5	22	10,6	Primary and Secondary Education	19	9,5	45	21,6
25-34 years old	79	39,5	96	46,2	High School	40	20	43	20,7
35-44 years old	22	11	60	28,8	University	129	64,5	102	49
45 years and older	26	13	30	14,4	Master's Degree and Above	12	6	18	8,6
Total	200	100	208	100	Total	200	100	208	100
Income (TL)	F	(%)	F	(%)	Profession	F	(%)	F	(%)
Those who did not specify income	55	27,5	60	28,8	Student	33	16,5	20	10
19,999 TL and below	46	23	55	26,4	Housewife	49	24,5	75	36,1
Between 20-29.999 TL	18	9	24	11,5	Public Employee	74	37	53	25
Between 30-39.999 TL	39	19,5	32	15,4	Private Sector Employee	40	20	47	22,6
40.000 TL and above	42	21	37	17,8	Self-employment	4	2	13	6,3
Total	20	100	208	100	Total	200	100	208	100
Marital Status	F	(%)	F	(%)					
Married	96	48	126	60,6					
Single	104	52	82	39,4					
Total	200	100	208	100					

The research data show a significant relationship between the demographic characteristics of olive and olive oil consumers in Hatay and Aydın and their usage preferences. These analyses support the validity of our research hypotheses and emphasise the influence of demographic criteria on consumer preferences.

PARTICIPANTS SHOPPING INFORMATION

Respondents in the two provinces were asked where they usually shop for olives and olive oil. This question is essential for businesses and consumers who sell olives and olive oil. The results are given in Table 4.

Table 4. Participants' Shopping Information-1

The Place Where Olive Oil Shopping is Done				
	HATAY		**AYDIN**	
Place of purchase	F	%	F	%
Direct Manufacturer	89	44,5	128	61,6
Other	19	9,5	61	29,5
Total	108	54	189	91,1
We Produce Ourselves	123	61,5	97	46,7
General Total	231	115,5	286	137,8

While oil production from one's garden is expected in Hatay, direct purchases from producers are preferred in Aydın. Moreover, the proportion of purchases from other sources is higher in Aydın than in Hatay. These results show the differences in consumer shopping preferences between the two provinces. This information can help shape marketing strategies for olive oil according to the respective regions. For example, in Hatay, direct communication with producers who produce their oil from their crops can increase sales, while in Aydın, online shopping platforms or chain markets can be encouraged. In addition, studies addressing these two regions can be conducted to examine why olive and olive oil shopping from chain markets and other platforms is not done or remains weak, especially in Hatay, and studies that build trust in consumers and the people of the region can be provided.

Table 5. Participants' Shopping Information-3

The Most Important Criteria in Olive and Olive Oil			
HATAY		**AYDIN**	
CRITERIA	**TOTAL SCORE**	**CRITERIA**	**TOTAL SCORE**
Taste	937	Taste	818
Smell	827	Colour	804
Colour	762	Quality	725
Price	699	Price	724
Quality	591	Brand	664
Brand	588	Smell	633
Other	0	Other	0

The respondents were asked to rank the criteria that affect them when purchasing olives and olive oil in order of importance, and the answers were subjected to scoring using the ordinal ranking method. The results of the statements are given in Table 5. The most important criteria for purchasing olives and olive oil in Hatay:

- Taste and Smell Priority: Taste and smell were among the important criteria in both provinces. Dec. This shows that olive and olive oil consumers emphasize the taste and smell of products, and this is the critical factor.
- Colour and Quality: Color and quality are also among the essential criteria. This reveals that consumers attach importance to the visual fascination and quality of the products.
- Price and Brand: Price and brand statements are essential to attracting consumers' attention. However, they rank behind factors such as taste, smell, colour, and quality.

This table highlights certain differences in consumers' preferences when purchasing olives and olive oil in Hatay and Aydın provinces. For example, taste, smell and color are of critical importance in Hatay, while taste, color and quality come to the fore in Aydın. These differences may be due to the cultural and geographical characteristics of both provinces, as well as the habits of consumers.

Table 6. Participants' Shopping Information-4

Preferred Brands for Olive and Olive Oil Shopping		
Brand	**HATAY**	**AYDIN**
My Own Oil	79	50
Komili	30	33
Tariş	2	49
Yudum	7	12
Kırlangıç	1	13
Other	81	51
Total	200	208

This table compares consumers' olive oil brand preferences in the two provinces. Table 6 gives the findings obtained when the brands preferred by consumers in Hatay and Aydın provinces are compared.

- Own Oil and Other Brands: In both provinces, consumers prefer oil produced from their crops. This indicates that they attach importance to local production and naturalness. In addition, the choices in the Other category are also noteworthy and include local producers and different brands. In other options, consumers prefer domestic brands such as Kaplan, Sızma, Sırma, Sidal, Oleamea, Marmarabirlik, Aktepe Birlik, Aydoğmuş, Egem, Nova Vera and Eti. In this respect, factories, direct producers and different brands are among the prominent preferences in both provinces.
- Brand Preferences: While the Tariş brand is more preferred in Aydın, Komili and other brands remain popular in Hatay. This shows that marketing and sales tactics, strategies and brand awareness are also important in addition to regional differences.

Moreover, differences in the brands consumers prefer may be due to factors such as product quality, product price, packaging design, product content or marketing campaigns. These results can be an essential guide for marketing professionals and business owners to understand local markets better and respond to customer needs.

COMPARISON OF OLIVE OIL USAGE AREAS OF OLIVE OIL CONSUMERS LIVİNG IN HATAY AND AYDIN

This part of the study compares the general uses of this crop by olive oil consumers who participated in the survey in Aydın and Hatay provinces. Table 7 shows the statements regarding the usage areas for the participants of both provinces and the answers of the participants. When the answers given by the participants to the statements are analysed, there is a difference of 0.36 between the averages of the participants (Hatay: 3.41, Aydın: 3.77) calculated by considering all statements. When Hatay and Aydın provinces are compared, the statements with the highest average in the olive oil usage areas of olive and olive oil consumers in Hatay province are listed as follows:

1. Olive oil is used in soap making (4,22 points)
2. Olive oil is used to repair hardened and challenging-to-open objects (4.08 points)
3. Olive oil is used to massage the body (4.06 points)
4. Olive oil is used for the treatment of fractures, dislocations and sprains in the body (3.99 points)
5. Olive oil is used in cosmetics (3.97 points)
6. Olive oil is used for frying (3.89 points)
7. Olive oil is used for diaper rash and redness (3,70 points)
8. Olive oil is used to repair rusted objects (3,30 points)
9. Furniture, wooden goods and decorative products are made from olive wood (3.09 points)

When Hatay and Aydın provinces are compared, the statements with the highest average in the olive oil usage areas of olive and olive oil consumers in Aydın province are listed as follows:

1. Olive oil is used to massage the body (4.52 points)
2. Olive oil is used in soap making (4.51 points)
3. Olive oil is used in cosmetics (4.48 points)
4. Olive oil is used for diaper rash and redness (4.43 points)
5. Olive oil is used to repair hardened and challenging-to-open objects (4.42 points)
6. Olive oil is used for the treatment of fractures, dislocations and sprains in the body (4.31 points)
7. Olive oil is used to repair rusted objects (3.92 points)
8. Furniture, wooden goods and decorative products are made from olive wood (3.71 points)
9. Olive oil is used to polish wooden objects (3.46 points)
10. Olive oil is used for cleaning furniture (3.2 points)
11. Olive oil is used for frying (3.02 points)

When both lists are examined, 9 of the 13 statements in the survey questions are expected. When the usage areas of olive oil in both provinces are examined, it is noteworthy that this product is used in cosmetics, health, food, cleaning, and especially in the production, maintenance, and repair of wooden products. The participant responses in Table 7 show that olive and olive oil usage is versatile and clearly expresses the trendy usage areas of this product in recent periods.

Table 7. Participants Responses to the Statements Related to the Uses of Olive Oil

HATAY			**AREAS OF USE**	**AYDIN**		
N	Mean	St. Deviation		**N**	Mean	St. Deviation
200	3,70	1,544	Olive oil is used in diaper rash and redness.	**208**	4,43	1,144
200	4,06	1,366	Olive oil is used for massaging the body.	**208**	4,52	1,103
200	2,68	1,692	Olive Kernel is used to make the rosary.	**208**	2,13	1,459
200	3,89	1,576	Olive oil is used in frying.	**208**	3,02	1,806
200	2,58	1,624	Olive oil is used to polish wooden objects.	**208**	3,46	1,75
200	2,08	1,495	Olive oil is used for cleaning furniture.	**208**	3,2	1,838
200	2,71	1,792	Olive kernel is used as heating fuel.	**208**	2,99	1,767
200	4,22	1,348	Olive oil is used in soap making.	**208**	4,51	1,138
200	3,97	1,387	Olive oil is used in cosmetics.	**208**	4,48	1,076
200	3,30	1,692	Olive oil is used to repair rusted objects.	**208**	3,92	1,595
200	4,08	1,408	Olive oil is used to repair hardened and challenging-to-open objects.	**208**	4,42	1,169
200	3,09	1,708	Furniture, wooden goods and decorative products are made from olive wood.	**208**	3,71	1,669
200	3,99	1,384	Olive oil treats fractures, dislocations and sprains in the body.	**208**	4,31	1,267
	3,41		**TOTAL**		**3,77**	

1: I never use it, 2: I do not use it, 3: No idea, 4: I use it, 5: I always use it

COMPARISON OF OLIVE OIL CONSUMERS' PERCEPTIONS OF OLIVE OIL LIVING IN HATAY AND AYDIN

In this part of the study, statements about olive oil for olive oil consumers in Hatay and Aydın provinces participating in the survey are included, and these statements are compared in general. Single Factor Multiple Variance Analysis (MANOVA) was applied to determine whether there is a difference between the responses of olive and olive oil consumers in both provinces to the statements about olive oil. The results of the MANOVA analysis and Hotelling's T-test are shown in Table 8.

In Hatay and Aydın provinces, there is a significant difference in participation in the statements related to olive oil for consumers of olive oil. There are significant differences in the following statements:

1. I think that olive oil deserves the money I pay (<,001)
2. Packaging is essential when buying olive oil (<.001)
3. Extra virgin olive oil has the highest nutritional value (<.001)
4. I trust branded olive oil more (<.001)
5. Mosquitoes do not bite skin smeared with olive oil (<.001)
6. Olive oil is good for chronic constipation and has a protective effect on the stomach (<.001)
7. Olive oil regenerates cells and protects against cancer (<.001)

Table 8. Comparison of Responses to Statements Related to Olive and Olive Oil in Hatay and Aydın Province

Statements	**Means**			**MEANINGFU**
	HATAY	**AYDIN**	**F VALUE**	**LNESS LEVEL**
I think olive oil prices are affordable.	2,17	2,09	0,335	0,563
I think olive oil deserves the money I pay for it.	2,71	2,18	11,275	<,001
I think expensive olive oil is of better quality.	2,46	2,64	1,366	0,243
Perfect olive oil burns the throat when drunk.	3,78	4,08	5,690	0,018
Packaging is important when buying olive oil.	3,59	4,23	20,203	<,001
Extra virgin olive oil has the highest nutritional value.	3,93	4,35	14,278	<,001
I trust branded olive oil more.	2,64	4,00	76,407	<,001
It is necessary to buy quality products from familiar producers.	4,64	4,80	4,122	0,043
Olive oil nourishes the skin, strengthens the nails and nourishes the hair.	4,73	4,88	5,434	0,020
Mosquitoes do not bite the skin smeared with olive oil.	3,37	4,21	41,839	<,001
Olive oil is good for chronic constipation and has a protective effect on the stomach.	4,49	4,83	18,918	<,001
Olive oil regenerates cells and protects against cancer.	4,41	4,79	19,315	<,001
Dishes made with olive oil are more delicious than other oils.	4,74	4,84	2,808	0,095
Hotelling's T Test F Value = 10,596 Degrees of Freedom = 13 Level of Significance = <.001				

It is seen in Table 8 that Hotelling's T-test result is significant (Significance Level <.001). Accordingly, it can be said that the perceptions of olive and olive oil in Hatay and Aydın provinces are different from each other as a whole. Olive and olive oil consumers in Aydın believe more in the various benefits of olive oil and have more trust in branded products. In addition, consumers in Aydın give higher scores on issues such as the care of the packaging and the perception of the quality of olive oil's ability to burn the throat. On the other hand, Olive and olive oil consumers in Hatay are less convinced that olive oil offers value for money and have lower satisfaction rates. At the same time, Hatay olive oil consumers, who are aware of olive oil's nutritional value and health benefits, show high confidence in buying a quality product from a familiar producer. This shows high trust in local producers and the tendency to prefer local products. Satisfaction with the price of olive oil and its value for money is also not high in Aydın, but there is a more pronounced perception of the relationship between price and quality of olives and olive oil. The belief that expensive olive oil is better quality is more common in Aydın than in Hatay. These data show that olive and olive oil consumers in both provinces have different judgments and priorities regarding olive oil, and these differences should be considered when marketing strategies are being developed.

When the information sources that olive and olive oil consumers in the two provinces most trust and attach importance to in their product purchase decisions are analysed, it is seen that 55.6% of the respondents consider their family as the most trusted source of information. This is because the majority of families are olive oil producers, so since they know and consume class A olive oil, there is usually more confidence in the choice of

families. This emphasises that family plays a vital role in consumers' decision-making process. Expert Opinion was identified as the second most reliable source of information by 29.9% of the respondents. The majority (50.7%) of the information sources used by consumers during product research show that they obtained the information for product research from their families. This indicates that consumers prefer to get information by consulting their families before purchasing. Expert Opinion ranked second with 28.7%. Regarding the first source of information that Olive and Olive Oil consumers use to draw attention to products, 49% of them get information about the product from their families. These tables show us that the family is essential in attracting consumers' attention, even though social networking platforms are perceived to be ahead. In the second place, Expert Opinion maintains its place at 25%. The answers to the statements reveal that family and expert opinions play an essential role in the product purchase phase of Olive and Olive Oil users. These findings are essential to determine marketing strategies and consumer relationship management. In addition, the fact that product promotion and marketing strategies are family-oriented shows that consumers can be more effective in decision-making.

Table 9. Accepted and Rejected hypotheses

	HYPOTHESES	**STATUS**
H1	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that olive oil prices are affordable.	REJECTED
H2	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that olive oil deserves the money paid for it.**	**ACCEPTED**
H3	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that expensive olive oil is of better quality.	REJECTED
H4	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that perfect olive oil will burn the throat when drunk.	REJECTED
H5	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that packaging is essential when buying olive oil.**	**ACCEPTED**
H6	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that extra virgin olive oil has the highest nutritional value.**	**ACCEPTED**
H7	**There is a significant difference between consumers in Hatay and Aydın in terms of trusting branded olive oil more.**	**ACCEPTED**
H8	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that it is necessary to buy quality products from familiar producers.	REJECTED
H9	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that olive oil nourishes the skin, strengthens the nails and nourishes the hair.	REJECTED
H10	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that mosquitoes will not bite the skin smeared with olive oil.**	**ACCEPTED**
H11	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that olive oil is good for chronic constipation and has a stomach protective effect.**	**ACCEPTED**
H12	**There is a significant difference between consumers in Hatay and Aydın in terms of thinking that olive oil regenerates cells and protects against cancer.**	**ACCEPTED**
H13	There is a significant difference between consumers in Hatay and Aydın in terms of thinking that dishes made with olive oil are tastier than other oils.	REJECTED

Table 10. Responses of consumers in Aydın and Hatay provinces to statements about their preferences for Attraction, Purchase and Research sources

Olive and Olive Oil Consumers' Preference for Introductions Sources.						
OLIVE OIL						
	Purchasing		**Research**		**Attracting Attention**	
Information Source	F	%	F	%	F	%
Advertisement	4	1,0	10	2,5	25	6,1
Sales Person	6	1,5	7	1,7	6	1,5
Introduction	11	2,7	13	3,2	21	5,1
Friends' Advice	38	9,3	54	13,2	54	13,2
Family	227	55,6	207	50,7	200	49,0
Expert Opinion	122	29,9	117	28,7	102	25,0
TOTAL	408	100	408	100	408	100

CONCLUSIONS AND RECOMMENDATIONS

This research was conducted among consumers in Hatay and Aydın provinces, the two most crucial olive and olive oil regions. The research focuses on comparing the two provinces in terms of olive oil usage and perceptions. In the two provinces, in-depth and face-to-face interviews were first conducted on consumers and their preferences, and answers were obtained from experts in this field to question which areas the crop is used mainly. The questions were enriched with the findings obtained from the experts and the literature studies, and a 5-point Likert scale was used in the survey study with the idea that the participants could respond better and the measurement could be healthier.

The results and recommendations obtained in this study can be listed as follows:

1. When the purchasing behaviour of olive and olive oil consumers in the two provinces is examined, there is a general dissatisfaction among consumers, especially regarding the price corresponding to the quality of this product, and at the same time, olive oil is mainly bought directly from the producer in both provinces, or it is the consumers' product. They do not prefer to buy from retail outlets, and they emphasise their distrust of these places. There is an inverse relationship between this view of consumers and the fact that retail outlets are more popular among the new branded sellers in this field. Regarding price stability, it is concluded that consumers in both provinces give more importance to the first three most essential criteria of the product, which are Taste, Smell and Color, as well as the conditions in how and how the product is produced, price and brand are the last essential criteria. If a product has the first three criteria, they will continue to consume it regardless of the price.

- In this sector, producers and sellers can gain consumers' trust by transparently presenting all the production process details to consumers. In addition, by allowing consumers to try products on a minimal scale, they can ensure that they understand the value of quality products. Developing products by giving importance to customer satisfaction and taking feedback into account creates a positive perception in consumers and ensures loyal customers. In addition, maintaining price stability and conducting campaigns, especially for low-income groups, will enable sellers to gain a strong position in the market.

2. Consumers in the two provinces use olives and olive oil primarily in food, health, cleaning, and cosmetics, and it is seen that they produce and consume all kinds of products for this crop's whole body and fruit. While the prevalence of the healing effect of this product in Hatay province, especially in cases such as falls, sprains, dislocations or fractures in the field of health, is less known in Aydın province. While comparing these two provinces in the general subject of the research, expanding the areas of use in these two provinces and other provinces and enriching the market of this product can lead individuals to a healthier and more conscious consumer profile. The areas mentioned above of use still maintain and improve their effectiveness. The marketing world can provide consumers with a broader range of uses for this product.

- This situation offers essential opportunities in the marketing world. First, the areas of use of olives and olive oil can be introduced to consumers through information and various local events. By making agreements with influencers and experts, content can be created that introduces the areas of use of the product, and all segments can have access to the areas of use related to this product. By creating small samples and distributing free trial products to consumers through various trainings, especially local events, consumer interest can be attracted, thus increasing diversity in regions that do not know the usage area of olive and olive oil and creating a solid position in the market.

3. Advertisements, promotions, expert opinions from salespeople, etc., are sources of information that consumers use in their research. Olives and olive oil have started to attract attention thanks to the culinary trends spread over the internet. This has popularised the use of olive oil and increased consumer demand for these products. However, although information sources such as advertising and promotion, which are effective in attracting attention, emphasise visuality, the family element or expert opinions, which are classical sources of research, information, purchasing and attracting attention, are still among the sources that maintain their importance. Some information may still be undiscovered or, even if discovered, may not have seen the light of day.

- Especially in this regard, marketers can emphasise the value of this product among families by creating campaigns aimed at families and gaining their support. By collaborating with family members who produce and consume their own olives and olive oil, consumer loyalty can be gained by creating content that emphasises the importance of family in information sources such as advertising and social media.

Marketers, advertising companies, and research companies can expand their efforts to reach this golden information waiting to be discovered and create a wide range of uses for this product.

As a result, much different information and results can be obtained when this research is applied in different periods and cities. The findings from the two cities were shared with the participants in this research, and the findings mutually influenced the participants and created new experiences and uses. New information and usage areas can be reached when this research is applied in other cities favouring olives and olive oil.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

All authors have read and accepted the final version of the article. The authors confirm that the Text, Figures and Tables are original and have not been published before.

Ethics committee approval

Permission of the Ethics Committee was granted by the Social and Humanities Ethics Committee of Kahramanmaraş Sütçü Imam University with Date: 28.06.2024 Reference Number: 322405 Approval No: 2.

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Isolation of phosphate solubilizing bacteria from different medicinal aromatic plants and identification using MALDI TOF MS

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DergiPark AKADEMİK

Abstract

Phosphate-solubilizing bacteria, which are among the plant growth-promoting bacteria, dissolve insoluble phosphate in the soil by several pathways and promote plant growth. Therefore, it offers an alternative option instead of applying chemical fertilizers that disrupt soil chemistry and ecological balance. Although research on phosphate solubilizing bacteria has increased recently, the research on the peppermint and fennel rhizosphere is still limited. Investigating different rhizospheric local bacteria that can solubilize phosphate and replace chemical fertilizers is necessary. It was determined that 15 of the 53 bacterial isolates obtained from peppermint (*Mentha piperita* L.) and fennel (*Foeniculum vulgare* L.) rhizospheres formed a transparent (halo) region around the colonies on Pikovskaya Agar (PKA) medium using the MALDI-TOF MS method. The morphological, biochemical and IAA production of these isolates as well as quantitative measurements of phosphate solubilization by the isolates in NBRIP broth medium was evaluated. The highest efficiency was noted from *Bacillus subtilis* MMS-7 with solubilization value of 281.6 mg L^{-1}. This was followed by *Pseudomonas fluorescens* MMS-11 with solubilization value of 263.4 mg L^{-1} and *Bacillus thuringiensis* MMS-3 with solubilization value of 172.1 mg L^{-1}, respectively. Among the Phosphate solubilizing bacterial isolates, P solubilization index ranged 1.2-3.7 on PKA agar medium. Additionally, the highest IAA production was noted at 23.38 µg ml^{-1}, using *Bacillus subtilis* MMS-7. This was followed by *Pseudomonas fluorescens* MMS-11 with value of 19.72 µg ml^{-1} and *Bacillus thuringiensis using* MMS-3 with value of 18.98 µg ml^{-1}. This study demonstrated that selected local isolates can be used as effective phosphate-based microbial fertilizers.

Keywords: *Mentha piperita*, *Foeniculum vulgare*, phosphate solubilizing bacteria, MALDI TOF MS

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INTRODUCTION

Phosphorus (P) is one of the primary macronutrients that limits plant growth after nitrogen. Phosphorus, found in biomolecules like nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids, play a role in many cellular processes, especially photosynthesis and cell division. It is essential for biochemical and physiological processes such as root development, flower and seed formation, and nitrogen fixation. Moreover, phosphorus is an important element that plant gain resistance against diseases. Phosphorus makes about 0.2 % - 0.8 % of a plant's dry weight. The healthy growth of a plant depend on the amount of phosphorus in the soil, along with other elements (Li et al., 2023). The abundance of phosphorus in the soil does not mean that the plant can use it. Most soils contain significant amounts of P, but a large proportion of it is bound to soil components. Phosphorus, a reactive element, is not found in elemental form in the soil, but is found as insoluble inorganic phosphorus and organic phosphorus. Plants can't use this insoluble phosphorus directly. Plant cells absorb and utilize a significant amount of the phosphorus in the soil in the form of phosphate ions. Chemical phosphorus fertilizers applied to agricultural land are immobilized by cations like Ca^{+2}, Al^{+3}, and Fe^{+3}, turning them into insoluble forms that

plants can't use. However, some microorganisms in the soil can change this situation in favor of plants (Pan & Cai, 2023). Microorganisms play a significant role in the phosphorus cycle. They enrich the soil with phosphorus by hydrolyzing insoluble compounds into organic and inorganic phosphorus compounds. Therefore, they naturally operate as a phosphorus reserve without harming the environment. Among the phosphorus-solubilizing microorganisms (*Bacillus* spp., *Pseudomonas* spp., and *Rhizobium* spp.), fungal genera (Penicillium and Aspergillus), actinomycetes, and arbuscular mycorrhizal (AM) fungi are very important (Thampi et al., 2023).

Phosphate-solubilizing bacteria in agriculture enhance plant growth and improve nutrient uptake. These bacteria solubilize phosphorus in the soil and make it available to plants, to facilitate their increased growth and development. Furthermore, the developing plants gain increased resistance to various abiotic stresses (drought) and biotic stresses (diseases). Numerous studies carried under in vitro conditions confirm that the phytochemicals and secondary metabolites found in peppermint (*Mentha piperita*) and fennel (*Foeniculum vulgare*) have antibacterial and antiviral characteristics (Patil et al., 2023; Rafieian et al., 2024). Despite their importance in medicine, pharmacy and perfumery, studies on the phosphate solubilizing bacteria are limited and their impact on agricultural productivity is not reaped in a meaningful way (Cheng et al., 2023). The environmental and health hazards posed by chemical fertilizers, coupled with their soaring costs, underline the need for use of sustainable methods during cultivation of medicinal and aromatic plants like peppermint and fennel. Consequently, the importance of alternative, eco-friendly fertilizers has been brought to the forefront by these concerns.

Bacterial identification techniques rely on culture media and tests assessing morphology, physiology, and biochemistry, typically requiring 3–5 days and expert skills. Additionally, nucleic acid-based methods like DNA-DNA hybridization, 16s rRNA, G+C ratio, RT-PCR, and fluorescent in-situ hybridization have been developed. Nonetheless, these methods are expensive, slow, and require significant expertise. Thus, there is a need for rapid and accurate identification methods to address bacteria and help isolate novel environmental strains. MALDI TOF MS offers advantages over traditional microbiological methods, including reliability, speed, simplicity, cost-effectiveness, and ease of use (Ashfaq et al. 2022). This study aimed to identify the local bacterial strains in the fennel and peppermint rhizosphere using MALDI TOF MS protocol and evaluate their potential as a phosphate solubilising microbial fertilizer.

MATERIALS AND METHODS

Collection of Soil Samples

Rhizospheric soil samples were collected in May 2024 from 2 different medicinal plants peppermint (*Mentha piperita* L.) and fennel (*Foeniculum vulgare* L.) in the Medicinal Plants Garden of the Department of Field Crops, Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E), Ankara University, Türkiye. The soil samples were promptly collected from the rhizospheric soil of these plants at a depth of 10 cm, and stored in sterile plastic bags, which were transferred to the laboratory for further analysis. Figure 1 shows the flowchart of the process from isolating bacteria in the medicinal plants' rhizospheric soil sample to determine their phosphate solubilizing abilities.

Isolation of Phosphate Solubilizing Bacteria

The bacterial isolates were tested for their ability to solubilize phosphate according to the methods of Pikovskaya (1948) using PKA medium. Phosphate solubilizing bacteria were isolated from 1 g of rhizospheric soil samples using the serial dilution method. The soil samples were homogenized in 10 ml of sterile isotonic saline water. The soil samples (1 g) were mixed with 9 ml of 0.85% saline (NaCl) sterile water and then homogenized in a shaker for 10 min. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6}. These dilutions were spread on Pikovskaya's Agar (PKA) (0.2 g L^{-1} NaCl, 10 g L^{-1} glucose, 0.2 g L^{-1} KCl, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.002 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.5 g L^{-1} yeast extract, 0.002 g L^{-1} $MnSO_4 \cdot H_2O$, and 1000 ml distilled water) and incubated for 5 days at 30 °C. The formation of a clear halo zone around the colonies on PKA agar plates indicated the presence of phosphate solubilizing bacteria. Single colonies with clear zones were subcultured to obtain pure cultures. Pure phosphate solubilizing bacterial colonies were spot inoculated at the center of the Pikovskaya agar medium. After 10 days of incubation at 30 °C, the zones of phosphate solubilization around the colonies were measured. The experiments were performed in triplicate. The purified isolates were maintained on nutrient agar plates at 4 °C, and the duplicates of each isolate were preserved in 40% glycerol stocks at -80 °C. The solubilization index (SI) was determined using measurements taken after seven days of growth from a point inoculation on PKA medium at 28 °C (Meena et al., 2015).

$$\mathrm{SI} = \frac{\text{Colony diameter} + \text{Halo zone diameter.}}{\text{Colony diameter}}$$

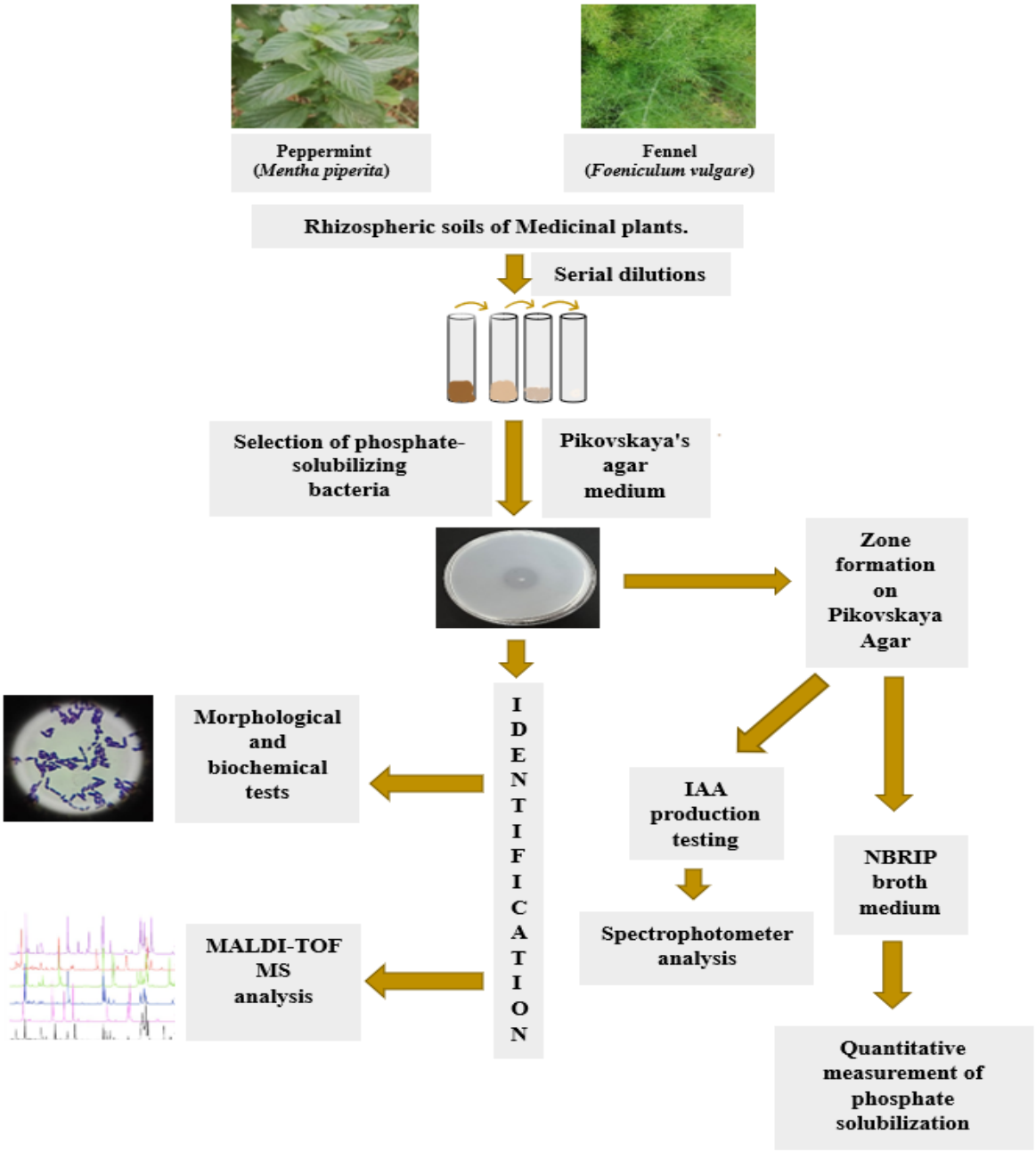


Figure 1. Process flowchart for identifying isolates in rhizospheric soil samples

Quantitative Measurement of Phosphate Solubilization

The phosphate solubilizing efficiency of 15 phosphate solubilizing bacteria that previously created a transparent (halo) zones on Pikovskaya Agar was assessed using the methodology developed by Barton (1948). NBRIP broth (5 g L^{-1} $Ca_3(PO_4)$, 2.5 g L^{-1} $MgCl_2.6H_2O$, 10 g L^{-1} glucose, 2.25 g L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} $(NH_4)_2SO_4$, and 0.2 g L^{-1} KCl) was used to quantify the phosphate solubilizing ability of phosphate-solubilizing isolates. For this purpose, 0.1 ml of fresh isolate (10^8 CFU ml^{-1}) was inoculated in triplicate into test tubes containing 10 ml of NBRIP growth medium and incubated at 30 °C at 180 rpm for 7 days. After incubation, the tubes were centrifuged at 5000 rpm for 10 min, and then the supernatant of each culture was analyzed for phosphate concentration in mg L^{-1}. The experiments were performed in triplicate. Non inoculated medium was used as control.

Morphological and Biochemical Characterization of Bacterial Strains

The morphological characterization of phosphate solubilizing bacteria was carried out using color, motility, and Gram staining tests. The biochemical characterization was performed using catalase and oxidase tests. The catalase and oxidase test of the isolates was determined according to the protocol described by Clarke and Cowan (1952). Two drops of 30% hydrogen peroxide were dropped on the colonies taken with a sterile loop and the

emergence of gas bubbles was observed after the catalase test. The observation of gas bubbles indicated a positive result. For the oxidase test, 1% tetramethyl-p-phenylenediamine was dropped on the colonies using a sterile loop, and a change in color to blue indicated a positive result.

Identification of Phosphate Solubilizing Bacteria

MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) was used for bacterial identification. Microorganisms were identified by their unique molecular fingerprints by the MALDI Biotyper CA System. Protein profiles of microorganisms' biomolecules (such as protein, peptide, sugar, and polymer) are ionized and then passed through an electric and/or magnetic field in this method. The profile spectra are compared graphically with reference microorganisms in the system's database to accurately identify them at genus and species level (Sivri & Öksüz, 2019).

Determining IAA Production by Phosphate-Solubilizing Bacteria

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. Bacterial cultures were cultivated for 48 hours at 30±2 °C. These were centrifuged for 30 minutes at 3000 rpm. The supernatant (2 ml) formed after centrifuge was mixed with 4 ml of the Salkowski reagent (50 ml, 1 ml 0.5 M $FeCl_3$ solution, 35% perchloric acid) and two drops of orthophosphoric acid. Pink appearances indicated the presence of IAA. The presence of IAA in the culture supernatant was determined spectrophotometrically (SHIMADZU UVmini-1240 Spectrophotometer) at 530 nm.

Data Analysis

Data on phosphate solubilization efficiency and solubilization index (SI) were analyzed in triplicate with JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean ± Standart Devision (SD) (Genç & Soysal, 2018).

RESULTS AND DISCUSSION

Identification of Isolates

Out of the 53 isolates taken from the rhizospheres of 2 different medicinal plants, it was determined that 15 of them created a clear zone around the colonies including 5 Bacillus (*B. thuringiensis* MMS-3, *B. subtilis* MMS-7, *B. megaterium* MMS-8, *B. simplex* MMS-10, *B. simplex* MMS-12), 3 Pseudomonas (*P. lutea* MMS-4, *P. fluorescens* MMS-11, *P. boreopolis* MMS-13), 1 *Pantoea* (*P. agglomerans* MMS-15), 1 Flavobacterium (*F. hydatis* MMS-1), 2 Stenotrophomonas (*S. rhizophila* MMS-9, *S. rhizophila* MMS-14), 1 Acinetobacter (*A. baumanni* MMS-6), 1 Enterobacter (*E. cloacae* MMS-5), and 1 Rhizobium (*R. radiobacter* MMS-2) on Pikovskaya Agar (PKA) medium in this study. These isolates were selected for morphological, biochemical and phosphate quantification. The distribution of bacterial species in plants according to MALDI-TOF MS results is given in Table 1. Percentage distribution of phosphate-solubilizing bacteria in peppermint and fennel rhizosphere is given in Figure 2.

Table 1. Bacterial species distribution according to MALDI-TOF MS result in plants

Plant	Number of species	Phosphate solubilizing bacteria identified by MALDI TOF -MS	NCBI No
Foeniculum vulgare L.	6	*Flavobacterium hydatis* MMS-1	991
		Rhizobium radiobacter MMS-2	362
		Bacillus thuringiensis MMS-3	1340496
		Pseudomonas lutea MMS-4	243924
		Enterobacter cloacae MMS-5	1328422
		Acinetobacter baumanni MMS-6	470
Mentha piperita L.	9	*Bacillus subtilis* MMS-7	1423
		Bacillus megaterium MMS-8	1404
		Stenotrophomonas rhizophila MMS-9	216778
		Bacillus simplex MMS-10	1478
		Pseudomonas fluorescens MMS-11	294
		Bacillus simplex MMS-12	1478
		Pseudomonas boreopolis MMS-13	86183
		Stenotrophomonas rhizophila MMS-14	216778
		Pantoea agglomerans MMS-15	549

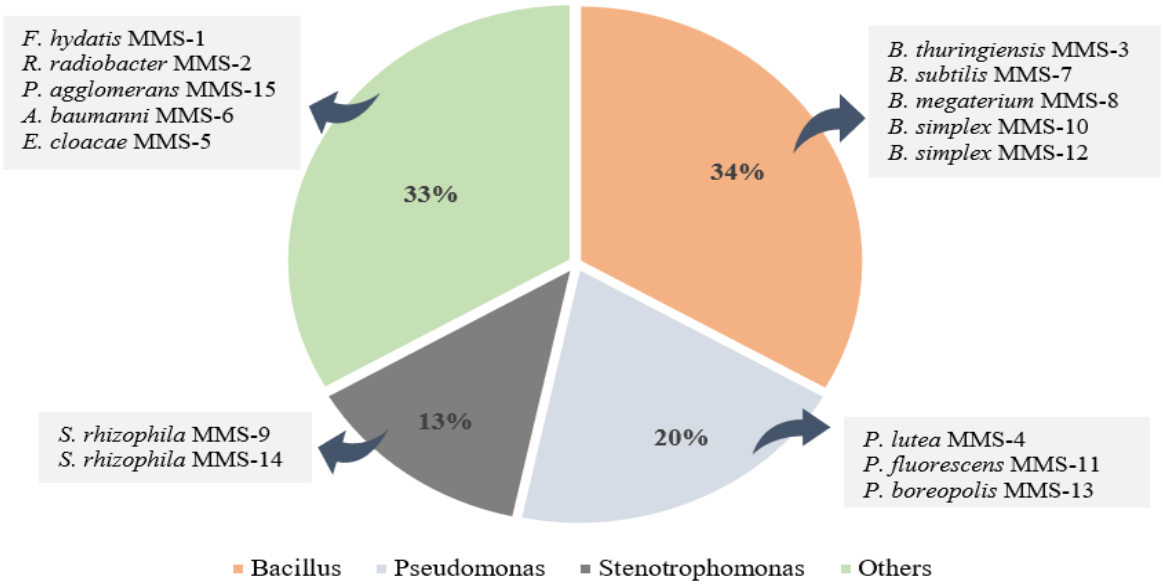


Figure 2. Percentage distribution of phosphate solubilizing bacteria by species

MALDI-TOF MS has become an invaluable tool for identifying bacteria down to the genus, species, and strain levels. Its recent rise in popularity can be attributed to its high precision and the speed at which it delivers results by MALDI-TOF MS to identify phosphate-solubilizing bacteria (Muthuri et al., 2012; Nazir et al., 2020). Çelikten & Bozkurt (2018) identified 120 bacteria from the wheat rhizosphere using MALDI-TOF to study plant growth-promoting bacteria. Bektaş (2021) used the MALDI-TOF MS method to identify 15 phosphate solubilizing bacteria isolated from 35 locations. Similarly, Öksel et al. (2022) identified bacteria in wheat rhizospheres using MALDI-TOF MS. The findings of this study revealed that Bacillus (34 %) pseudomonas (20 %) were the most common bacterial genera in *Mentha piperita* and *Foeniculum vulgare* rhizosphere. In a recent study, Dip et al. (2024) determined that the phosphate-solubilizing bacteria in the rhizosphere of *Sporobolus indicus* and *Panicum coloratum*, which were identified through the MALDI TOF method, predominantly belonged to the genera Enterobacter and Pseudomonas.

Morphological and Biochemical Characterization of Isolates

Five (5) out of the 15 isolates showed Gram (+) reaction in this study. The catalase test was positive for all isolates except for *F. hydatis* MMS-1, *B. subtilis* MMS-7, *S. rhizophila* MMS-9, and *S. rhizophila* MMS-14; whereas the oxidase test was positive for 6 isolates. Except *B. subtilis* MMS-7, motility tests all others samples were positive. Morphological and biochemical characteristics of phosphate-solubilizing isolates are given in Table 2.

IAA Production and Phosphate Solubilization Properties of Isolates

It was determined that a total of 6 isolates (MMS-7, MMS-11, MMS-3, MMS-9, MMS-14, MMS-15) from peppermint and fennel rhizospheres produced IAA in different amounts in this study. *Bacillus, Stenotrophomonas, Pseudomonas*, and *Pantoea* are among the identified genera of the IAA producing bacteria investigated in this study. The maximum IAA production was obtained in *Bacillus subtilis* MMS-7 with 23.38 µg ml^{-1} according to the findings of the current study. This was followed by *Pseudomonas fluorescens* MMS-11 with 19.72 µg ml^{-1} and *Bacillus thuringiensis* MMS-3 with 18.98 µg ml^{-1} (Figure 3A).

Indole-3-acetic acid (IAA), a primary auxin, is crucial for various plant processes from germination to maturity. It participates in cell division and expansion, leaf development, and the initiation and growth of roots, lateral roots, and root hairs. This phytohormone plays a vital role in communication between bacteria and their host plants. Bacteria producing IAA increase auxin levels, thereby boosting plant health and productivity (Ratnaningsih et al., 2023). According to Alemneh et al. (2021), 80% of bacteria isolated from the rhizosphere can produce IAA. IAA production has been determined in several PGPR genera, including *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, and *Pantoea*. Ahmed et al. (2014) reported that among 112 isolates isolated from different medicinal plant rhizospheres, 36 of them produced IAA and among them, *Pseudomonas fluorescens* Th98 and *Bacillus thuringiensis* Th100 strains produced IAA at the rate of 0.1 to 17 µg/100 ml. In the present study, *Pseudomonas fluorescens* MMS-11 (19.72 µg ml^{-1}) and *Bacillus thuringiensis* MMS-3 (18.98 µg ml^{-1}) produced IAA. Hassan et al. (2017) determined that endophytic *Bacillus cereus* Tp.1B isolated from the root of *Teucrium polium* L, a medicinal plant, produced IAA at a rate of 23.4 µg ml^{-1}. Shakeela et al. (2017) reported that *Bacillus subtilis* PkR34 isolated from the rhizosphere of a medicinal plant *Picrorhiza kurroa* produced IAA

at high levels (30.00 µg ml^{-1}). Similarly, the highest IAA producer (23.38 µg ml^{-1}) was *Bacillus subtilis* MMS-7 in the current study.

Table 2. Morphological and biochemical traits of phosphate-solubilizing bacteria

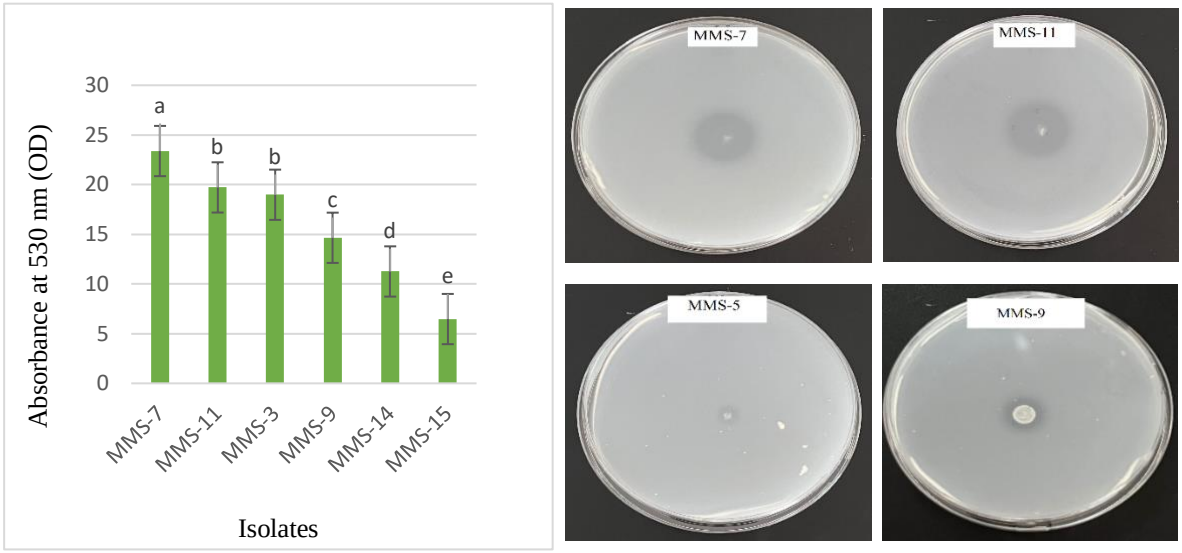
Isolates No	Gram Stain Test	Motility	Colony color	Biochemical Characteristics		
				Catalase	Oxidase	KOH 3%
Flavobacterium hydatis MMS-1	-	+	White	-	-	+
Rhizobium radiobacter MMS-2	-	+	White	+	+	+
Bacillus thuringiensis MMS-3	+	+	Cream	+	-	-
Pseudomonas lutea MMS-4	-	+	White	+	+	+
Enterobacter cloacae MMS-5	-	+	White	+	-	+
Acinetobacter baumanni MMS-6	-	+	Cream	+	-	+
Bacillus subtilis MMS-7	+	-	White	-	-	-
Bacillus megaterium MMS-8	+	+	White	+	-	-
Stenotrophomonas rhizophila MMS-9	-	+	White	-	+	+
Bacillus simplex MMS-10	+	+	Whitish	+	-	-
Pseudomonas fluorescens MMS-11	-	+	Cream	+	+	+
Bacillus simplex MMS-12	+	+	Cream	+	-	-
Pseudomonas boreopolis MMS-13	-	+	White	+	+	+
Stenotrophomonas rhizophila MMS-14	-	+	White	-	+	+
Pantoea agglomerans MMS-15	-	+	Cream	+	-	+

Note: * +, positive; –, negative

In previous studies, the researchers found similar results regarding IAA production from strains isolated from the rhizospheres of different medicinal plants. Liu et al. (2020) determined that *Bacillus subtilis* B9 strain isolated from sugarcane roots produced IAA and that B9 application increased sugarcane root development, root length, root weight and height in greenhouse experiments. Khan et al. (2020) reported that endophytic *Bacillus velezensis* Lle-9 isolated from *Lilium leucanthum* bulbs produced IAA at a rate of 23.2 µg ml^{-1}. Alaylar (2022) reported that endophytic Bacillus strains (ML-12, ML-36, ML-43, ML-46, ML-49, ML-55, ML-ML-59, ML-61, and ML-63) isolated from *Mentha longifolia* L. produced IAA.

Many studies have shown that *Pseudomonas* sp. obtained from the rhizospheres of different medicinal plants produces IAA (Thakur et al., 2017; Chandra et al., 2020). Al-Habib (2021) reported that *Pseudomonas putida* in the rhizosphere of *Eruca sativa*, which has medicinal properties, produced IAA and that the application of *P. putida* in greenhouse experiments increased rooting and germination. Wu et al. (2022) reported that *Pseudomonas fluorescens* BsEB-1 isolated from *Bletilla striata* root is an effective IAA producer. Similarly, it was determined that *Pseudomonas fluorescens* MMS-11 produced high concentrations of IAA in the current study. Marfungah et al. (2023) determined that the strain *Pseudomonas aeruginosa* RE81 produced the highest concentration of IAA among the PGPR strains isolated from the *Eucalyptus pellita* rhizosphere. Meneguzzi et al. (2024) determined that among 22 bacteria isolated from the rhizosphere of *Minthostachys verticillata*, a medicinal and aromatic plant, *Pseudomonas* sp. SM8 and *Pseudomonas putida* SM 21 produced high levels (556.7 ng/mL ve 717.8 ng/mL) of IAA in a recent study.

Shankar et al. (2011) stated that *Enterobacter* spp. and *Pantoea* spp. were among the plant growth-promoting bacteria predominantly found in the rhizosphere. Bose et al. (2016) reported that *Enterobacter cloacae* SN19 in the rizosphere of *Teramnus labialis* produced a high amount (382.23 µg ml^{-1}) of IAA. Abd el-Megeed and Youseif (2018) reported that *Pantoea* sp. NGB-W48 and *Pantoea* sp. NGB-W53 in the rhizosphere of the medicinal plant *Zygophyllum album* produced 164 and 127 µg ml^{-1} IAA, respectively. Panigrahi et al. (2020) reported that *Enterobacter cloacae* MG00145, isolated from the roots and stems of the medicinal plant *Ocimum sanctum*, produced IAA at a concentration of 17.807 µg ml^{-1}. On the other hand, *Enterobacter cloacae* MMS-5 did not produced IAA in the current study. According to Chiellini et al. (2023), Many *Stenotrophomonas* spp. are plant-probiotic bacteria that promote plant growth through different mechanisms. Vijayalakshmi and Senthilkumar (2023) determined that *Stenotrophomonas rhizophila*_SS11 was the most effective IAA producer among 86 isolates obtained from the rhizosphere of the medicinal plant *Tephrosia purpurea*. Similarly, *Stenotrophomonas rhizophila* MMS-9 and *Stenotrophomonas rhizophila* MMS-14 were determined to be effective IAA producers in the present study (Figure 3A).



A **B**

Figure 3. A. IAA production by isolates on NBRIP medium **B**. The transparent zones formed by phosphate solubilizing bacteria on PKA agar medium

Some bacteria, referred to as phosphate solubilizing bacteria (PSB), have the ability to dissolve both inorganic and organic forms of phosphorus in the soil. These bacteria employ various mechanisms to solubilize phosphate, predominantly through acid production. Consequently, they release phosphorus into the soil by means of their capacity to solubilize organic and inorganic phosphorus. Previous research has shown that the rhizosphere contains a substantially higher population of phosphate solubilizing bacteria than non-rhizospheric soil (Linu et al., 2019; Ibáñez et al., 2021). Moreover, According to Anand et al. (2016), phosphorus solubilizing bacteria (PSB) are more efficient than fungal species in the solubilization of phosphorus, and make up 1–50% of the total microbial population in soil. Siddique et al. (2021) mentioned that phosphate-solubilizing bacteria render phosphate available to plants by releasing various organic acids and enzymes, which solubilize the phosphate in the soil. P solubilization index of PSB strains are ranged 1.2 to 3.7 on PKA agar medium (Figure 3B). On the other hand, a rate of 67.8 to 281.6 mg L^{-1} was detected in the NBRIP broth medium. The highest value was obtained from *Bacillus subtilis* MMS-7 with 281.6 mg L^{-1} according to the phosphate solubilizing abilities of phosphate solubilizing bacteria in NBRIP broth medium. This was followed by *Pseudomonas fluorescens* MMS-11 with 263.4 mg L^{-1} and *Bacillus thuringiensis* MMS-3 with 172.1 mg L^{-1}, respectively. The phosphate solubilizing activities and solubilization index (SI) of the isolates are shown in Table 3.

Table 3. Phosphate solubilization activities and solubilization index (SI) of the isolates

Isolates No	**Phosphate solubilizing activity (mg L^{-1})**	**Solubilization index (SI) Mean ± SD**
Flavobacterium hydatis MMS-1	67.8± 2.28^{i}	1.2 ± 0,26^{f}
Rhizobium radiobacter MMS-2	135.9± 3.60^{e}	2.1±0,32^{cde}
Bacillus thuringiensis MMS-3	172.1± 3.96^{c}	3.1±0,26^{ab}
Pseudomonas lutea MMS-4	122.5± 3.55^{fg}	1.5±0,17^{def}
Enterobacter cloacae MMS-5	98.54± 3.53^{ijk}	2.2±0,60^{bcd}
Acinetobacter baumanni MMS-6	91.2± 1.47 ^{k}	1.3±0,2^{ef}
Bacillus subtilis MMS-7	281.6± 6.94^{a}	3.7±0,55^{a}
Bacillus megaterium MMS-8	107.8± 4.81^{hı}	2.3±0.1^{bcd}
Stenotrophomonas rhizophila MMS-9	158.7± 4.45^{d}	2.9±0,26^{abc}
Bacillus simplex MMS-10	95.9± 1.47^{jk}	1.6±0,26^{def}
Pseudomonas fluorescens MMS-11	263.4± 1.35^{b}	3.5±0,26^{a}
Bacillus simplex MMS-12	102.3± 2.69^{ij}	1.5±0,43^{def}
Pseudomonas boreopolis MMS-13	117.6± 2.48^{gh}	1.4±0,17^{def}
Stenotrophomonas rhizophila MMS-14	137.1± 1.04^{e}	2.4±0,2^{bcd}
Pantoea agglomerans MMS-15	131.9± 1.77^{ef}	1.9±0,36^{def}

*p<0,01; statistically significant level. a-l: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

Han et al. (2020) mentioned that *Bacillus*, *Enterobacter*, and *Pseudomonas* were the most common inorganic phosphate-solubilizing bacteria in the rhizosphere. Previous research indicated that Bacillus was an excellent phosphate solubilizer (Azaroual et al., 2020; Zhong et al., 2021). Mishra et al. (2016) reported that *Bacillus subtilis* NRCSS-II isolated from the rhizosphere of *Foeniculum vulgare* had phosphate solubilizing ability and when applied to the seed of this plant, provided yield increase up to 1744.35 kg ha^{-1}. In another study, Wang et al. (2020) reported that the *Bacillus subtilis* BPM12 strain in the corn rhizosphere dissolved phosphate with a ratio of 189.1 µg ml^{-1}. Gupta et al. (2022) determined that *B. subtilis* PS4, isolated from 3 different rice fields, was the strain with the highest phosphate solubilization efficiency of 50.9%. It was determined that *Bacillus subtilis* MMS-7 had a phosphate solubilization ability of 281.6 mg L^{-1} in NBRIP broth medium in the present study. ALKahtani et al. (2020) determined that *Bacillus thuringiensis* had a phosphate solubilization efficiency between 7.6 and 9.6 among 13 isolates they isolated from the rhizosphere of medicinal plants *Fagonia mollis* and *Achillea fragrantissima*. In the present study, the phosphate solubilizing index of *Bacillus thuringiensis* MMS-3 was determined as 3.1. Similar findings for *Bacillus thuringiensis* were reported by Ambreen et al. (2020) and Pantigoso et al. (2022).

It has been mentioned by Paul & Sinha, (2017); and Adhikari et al. (2021) that *Pseudomonas* spp. found in the rhizosphere are effective phosphate solubilizers and support plant growth. Amri et al. (2023) identified 28 phosphate-solubilizing bacteria in soil samples from various regions of Tunisia. They discovered that the solubilization index (SI) percentages of these bacteria ranged 2.14 to 3.51, with *Pseudomonas fluorescens* exhibiting the highest SI percentage. It was determined that the phosphate solubilization index in the Pseudomonas genus (*Pseudomonas boreopolis* MMS-13 and *Pseudomonas fluorescens* MMS-11) was 1.4-3.5 in the present study (Figure 3B). These results are in agreement with the findings of Roychowdhury et al. (2019), and Blanco-Vargas et al. (2020), which demonstrated that the solubilization index among various bacterial isolates, including *Pseudomonas* spp., ranged 2.56-4.50. They also showed that the formation of halo zones by these bacteria on growth plates is due to the production of organic acids, thus identifying them as effective phosphate solubilizers. Kaur et al. (2022) documented that 19 phosphate-solubilizing bacteria, isolated from the potato rhizosphere and identified using the MALDI-TOF-MS method, exhibited phosphate solubility ranging between 115 -747 µg ml^{-1}.

CONCLUSION

Phosphorus is crucial for plant metabolism and its non availability can significantly decrease crop yields. Around 67 % of agricultural soils are deficient in P, making it essential to address this issue. Chemical phosphorus fertilizers can disrupt soil fertility and cause eutrophication. Phosphate-solubilizing bacteria (PSB) can help eliminate phosphorus deficiency in soils. A study on 53 bacterial isolates from *Mentha piperita* and *Foeniculum vulgare* rhizospheres found such that *Bacillus subtilis* MMS-7 and *Pseudomonas fluorescens* MMS-11 were the most effective in phosphate solubilization. These bacteria can be used as cost-effective phosphate-solubilizing microbial fertilizers, contributing to sustainable agriculture. Further research is needed to assess their competence, plant growth performance, and antifungal effectiveness against various pathogens.

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.

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Foliar zinc sulfate application effects on biomass and forage traits of annual ryegrass (*Lolium multiflorum* Lam.) in zinc-deficient soils

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Abstract

Deficiencies of essential micronutrients in forage crops can result in reduced growth, diminished nutrient content, and reduced in forage quality. This can, in turn, affect the nutritional requirements of livestock and the overall productivity of the agricultural sector. The experiment was initiated during the 2023-24 production season and employed four zinc sulfate doses (0, 0.2, 0.4, 0.6 % w/v) and five varieties of annual ryegrass (*Lolium multiflorum* Lam. cv. İlkadım, cv. Master, cv. Baqueno, cv. Caramba, cv. Trinova), with four replications. SPAD measurements were obtained following the foliar application of zinc sulfate, and plant height (cm), flag leaf length (cm), flag leaf width (cm), leaf number per plant, and leaf area (cm^2) parameters were collected through the single mowing of annual ryegrass. After the fresh forage yield (t ha^{-1}) measurements, ADF (%), NDF (%), crude protein ratio (%), crude protein yield (kg da^{-1}) and relative feed value were measured and calculated. As a result of the data obtained, it was determined that foliar zinc sulfate applications can make positive changes in yield and quality, while at the same time increasing the amount of fiber. While İlkadım had the highest average value with 1.48 t da^{-1} in terms of hay yield, the highest value among zinc sulfate doses was obtained from 0.6 % with 1.47 t da^{-1}. High values were obtained at 0.4 % and 0.6 % doses. Among the varieties, İlkadım and Baqueno had higher yield and quality characteristics. However, it is understood that the responses to foliar zinc sulfate applications occurred in different percentages among the others.

Keywords: Foliar fertilizer, Zinc, Ryegrass, Forage quality, SPAD

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INTRODUCTION

Zinc (Zn) is a crucial micronutrient for plant growth and development, playing a significant role in various physiological and biochemical processes. Its importance is underscored by its involvement in enzyme activity, protein synthesis, and regulation of plant hormones, all of which are essential for optimal plant growth and yield. Zinc acts as a structural component and cofactor in numerous enzymes, facilitating critical metabolic pathways including those related to photosynthesis, respiration, and nitrogen metabolism (Broadley et al., 2007; Alloway, 2009).

Zinc deficiency is a widespread issue in agricultural soils, affecting crop yield and quality globally. It is estimated that zinc deficiency impacts a significant portion of the world's arable land, leading to reduced agricultural productivity and nutritional quality of crops (Alloway, 2009; Barman et al., 2018). For instance, in rice cultivation, zinc deficiency has been linked to lower yields, necessitating the application of zinc fertilizers to enhance crop performance (Rasel et al., 2023). Furthermore, zinc plays a critical role in synthesizing chlorophyll, which is essential for photosynthesis, and its deficiency can lead to chlorosis and stunted growth in plants (Khatun et al., 2018).

The mechanisms by which plants acquire and utilize zinc are complex. Zinc transporters, such as the Zn/Fe-regulated transporter family, are crucial for zinc uptake from the soil into plant cells (Khatun et al., 2018; Sinclair et al., 2018). These transporters are upregulated in response to zinc deficiency, highlighting the plant's adaptive

mechanisms to cope with low zinc availability (Khatun et al., 2018). Additionally, the interaction between zinc and plant growth-promoting bacteria has been shown to enhance zinc bioavailability and uptake, further supporting plant health and productivity (Jalal, 2024; Upadhayay et al., 2022).

Annual ryegrass (*Lolium multiflorum* Lam.) is increasingly recognized as an important forage crop due to its high yield potential, nutritional quality, and adaptability to various agricultural systems. Its significance is underscored by its role in mixed cropping systems, where it can enhance overall forage production and quality when combined with legumes such as red clover and berseem clover. Studies have shown that while the yield benefits from these mixtures may vary seasonally, annual ryegrass consistently contributes significantly to forage availability, particularly during the mid to late-growing season when clovers are slower to establish (Ryan-Salter & Black, 2012; Iuga et al., 2018).

The application of foliar zinc in annual ryegrass has been shown to significantly enhance both forage yield and quality. Studies have demonstrated that the application of zinc, particularly in the form of zinc sulfate, can lead to improved relative feed value (RFV) in forage crops, including annual ryegrass (Sher et al., 2022). Furthermore, highlighted that biofortification with zinc not only improves forage productivity but also enhances the nutritive value for livestock, making it a dual benefit for agricultural practices (Kumar & Ram, 2021). Additionally, emphasized that agronomic biofortification strategies, including foliar application of zinc, can effectively enhance the micronutrient content of forage crops, thereby improving their overall quality (Dhaliwal et al., 2022).

The increasing importance of annual winter forage crops as cover crops, catch crops and roughage sources has revealed the necessity of research for optimum cultivation of these crops. Annual ryegrass is one of these species. For this purpose, the effect of foliar applications on yield and quality in areas with zinc deficiency in ecologies with Mediterranean climate was investigated.

MATERIALS AND METHODS

Study site description

The experimental site is situated in Aydın Adnan Menderes University Research and Demonstration farms, Aydın within the Mediterranean climate zone (N: 37.762326°, E: 27.758774°). The area has mild and hot temperatures and plenty of sunlight. The long-term climate data for this location indicate that the average total precipitation was 573.7 mm and the average temperature was 13.92 °C during the months of cultivation. It should be noted that the year in which the experiment was conducted differed from the long-term average. In terms of monthly average temperatures, the period in which the experiment was conducted was notably warmer and drier than the long-term average. There were also significant differences between months in terms of precipitation totals. (Figure 1.)

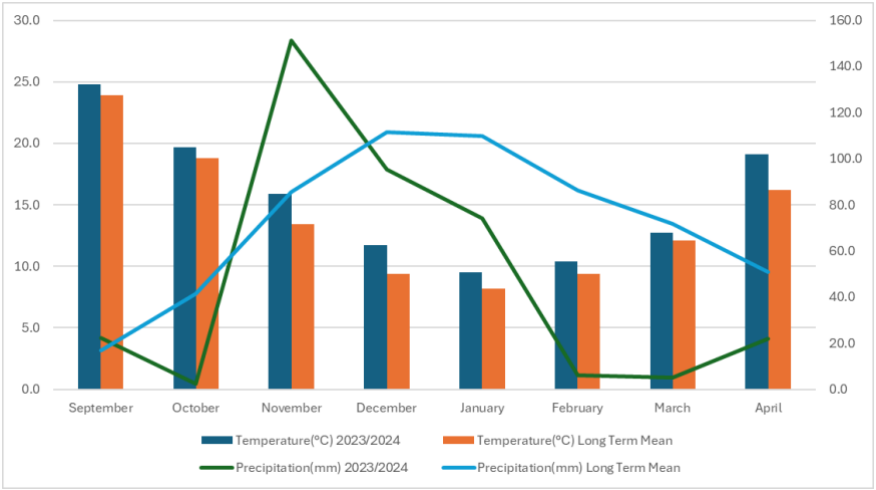

Figure 1. Monthly average temperature and precipitation values in the study area for 2023-2024 cropping season.

The studied soils at the site classified as inceptisols, the basic physical and chemical characteristics of which are as follows: had a pH of 8.09, soil organic matter of 11.4 g kg^{-1}, available phosphorus of 5.11 ppm, exchangeable potassium of 197 ppm, exchangeable calcium (Ca) of 4301 ppm, iron (Fe) of 14.94 ppm, zinc (Zn) of 0.35 ppm (deficient) and texture is sandy loam. Zinc deficiency is observed in soil with concentrations less than 0.5 ppm (Eyüpoğlu et. al., 1998).

Experimental Design

The experiment design was randomized complete block design (RCBD) with factorial arrangements in four replications conducted during the study years 2023/24. The plots were 1.2 meters in width and 5 meters in length, with six rows of plants, separated by 20 centimeters. The seeds were sown with a trial seeder in November 2023

at a depth of 3-4 cm and at a rate of 20 kg per hectare. The experiment utilized five annual ryegrass cultivars (*Lolium multiflorum* Lam. cv. İlkadım, cv. Master, cv. Baqueno, cv. Caramba, cv. Trinova) as the experimental material. Four different zinc sulfate doses (0-Control, 0.2, 0.4, 0.6% w/v) were applied to the plots of each variety. At each foliar application, an aqueous solution 0, 0.2, 0.4, 0.6% (w/v) of $ZnSO_4 \cdot 7H_2O$ ha^{-1} with 800 l ha^{-1} was sprayed in the very late afternoon until most of the leaves were covered. Harvesting was done with a self-propelled meadow mower (BCS-615 SL, Italy) at the flowering stage of the plants.

Measurements of annual ryegrass

Plant height measurements were made on 15 plants in each plot at harvest maturity. Aboveground biomass samples were collected from each plot at a stubble height of 2.5 cm. The samples were weighed and oven dried at 70 °C for 48 hours (Mikrotest Lab., Ankara, Türkiye). Using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Japan), the relative chlorophyll content of leaves was measured after (BBCH 63 stage) of foliar zinc sulfate treatments by taking measurements in the middle of 15 randomly chosen leaves per plot (Hoel, 1998). The leaf area measurement was carried out with the help of LI-COR 3000C (Lincoln, NE, USA) by measuring the whole leaf area of 15 samples taken homogenously from the plots before and after zinc sulfate application. Total N content was determined by the Kjeldahl method and multiplied by 6.25 to give crude protein content (AOAC, 2003). NDF and ADF contents were measured using the methods described by Van Soest et al. (1991) in an automated fibre analyzer (ANKOM 2000 Fibre Analyser, ANKOM Technology, NY, USA). Briefly, for NDF analysis, a total of 0.5 g of ground sample was placed in the fibre filter bag (F57, ANKOM Technology, NY, USA) and placed in the automatic fibre analyzer to digest the samples with dilute solutions of neutral detergent dry concentrate with triethylene glycol (FND20C, ANKOM Technology, NY, USA). The residues obtained after the determination of NDF can be digested directly with the solution of acid detergent concentrate - dry CTAB powder (FAD20C, ANKOM Technology, NY, USA) for the determination of ADF.

The crude protein yield (t ha^{-1}) and relative feed value were calculated using the obtained data and the procedures of Horrocks and Vallentine (1999).

Data analysis

All data were analyzed for homogeneity and normality using the Kolmogorov-Smirnov test. When significant differences were found via the ANOVA, means were compared using the Fisher's protected least significant difference (LSD) test at $p \leq 0.05$. The 'agricolae' package in R Studio v4 (Mendiburu and Mendiburu, 2019) was used for all one-way analyses, while the 'methane' package (Olivoto and Lucio, 2020) was run for Pearson correlation representations.

RESULTS AND DISCUSSION

The results of the ANOVA demonstrated that the annual ryegrass genotypes and different zinc doses had a significant impact on the plant traits under investigation. Significant differences were observed between the genotypes and the zinc doses in many parameters, including plant height, leaf number, flag leaf length, flag leaf width, fresh forage yield, and hay yield ($p < 0.01$). In particular, the effect of the zinc doses was found to be statistically significant in all of the aforementioned parameters. The interactions between genotypes and zinc doses were, in general, statistically significant. However, for some traits, this interaction was less significant ($p > 0.05$). This was particularly the case for ADF, and RFV parameters, where the genotype × zinc sulfate dose interactions were less significant. This indicates that zinc doses and genotypes alone were effective on these traits, but their interactions were limited. In contrast, in some other parameters, such as fresh forage yield, the genotypes x zinc dose interactions were significant. In these cases, the two factors affected plant performance (Table 1.).

Analysis of the mean values revealed the general tendency of the genotypes towards zinc sulfate doses. In terms of plant height, the highest mean value was observed at 0.4% dose (97.44 cm), followed by 0.6% (93.91 cm) and 0.2% (93.06 cm) doses. The control group had the lowest average plant height (89.85 cm). These results indicate that especially 0.4% dose had the most positive effect on plant height and zinc application significantly increased plant height. Considering the effects of zinc sulfate doses in different genotypes, striking results were observed on plant height. In the Master genotype, plant height increased with a peak (95.69 cm) at 0.4% dose, while it decreased at the higher dose of 0.6%. This indicates that increasing zinc doses do not increase plant height in a linear manner. On the other hand, İlkadım genotype reached the highest height especially at 0.6% dose (101.75 cm), indicating that it was positively affected by the increase in zinc concentration. The Trinova genotype reached the highest height at 0.4% dose (95.95 cm), but decreased at higher doses. Caramba genotype showed a balanced response, reaching the highest height value at 0.4% dose (100.80 cm). Baqueno genotype exhibited a relatively constant response at all doses and showed a limited response to high zinc concentrations.

When the number of leaves per plant was evaluated, the highest average number of leaves was obtained at 0.6% dose (6.29). This was followed by 0.4% (5.98) and 0.2% (5.87) doses, while the control group had the lowest number of leaves (5.32). This indicates that the number of leaves increased in direct proportion with increasing zinc sulfate doses and 0.6% dose promoted leaf growth. Differences were also observed in the number of leaves per plant between genotypes with zinc sulfate doses. Master genotype increased the number of leaves with increasing zinc concentration and reached the maximum value (6.15) at 0.6% dose. Ilkadim genotype reached the

maximum number of leaves (7.06) at 0.6% dose, indicating that it was positively affected by high zinc concentrations. In Trinova genotype, the number of leaves increased slightly as the dose increased and reached the highest value at 0.6% dose (6.21). The Caramba genotype showed a more limited response, peaking at 0.6% (5.97). The Baqueno genotype also responded positively to increasing zinc doses, reaching the highest leaf number at 0.6% (6.06).

Table 1. Combined analysis of variance for the effects of foliar zinc sulfate on the traits of annual ryegrass genotypes

SOV	df	Mean Squares						
		Plant Height	Leaf Number	Flag Leaf Lenght	Flag Leaf Width	Fresh Forage Yield	Hay Yield	Leaf Area
Genotypes	4	148.9**	1.35**	35.57**	5.29**	263.8**	150.7**	203.8**
Zinc Doses	3	194.4**	3.27**	108.64**	2.90**	610.4**	318.2**	72.8**
Genotypes × Zinc Doses	12	22.7**	0.14^{ns}	19.75**	0.37**	40.7**	16.8*	21.9**
	df	SPAD	ADF	NDF	CPR	CPY	RFV	
Genotypes	4	47.0**	8.57**	4.91**	0.95**	3443.4**	91.4**	
Zinc Doses	3	56.0**	13.89**	31.53**	22.03**	20493.0**	363.0**	
Genotypes × Zinc Doses	12	14.0**	2.02^{ns}	2.55*	0.55**	658.3**	30.0^{ns}	

** p<0.01, *p<0.05, ns: non-significant

In the flag leaf length averages, the highest value was obtained at 0.6% dose (57.75 cm), followed by 0.4% (56.95 cm) and 0.2% (54.65 cm). The flag leaf length of the control group had the lowest average (52.60 cm). These data show that especially high zinc doses have a positive effect on flag leaf length and the 0.6% dose provides the highest increase in this parameter. When evaluated in terms of flag leaf length, it was observed that as zinc concentration increased in Master genotype, the flag leaf length increased and reached a maximum at 0.6% dose (59.00 cm). İlkadım genotype gave the highest response to zinc application and reached the longest flag leaf length at 0.6% dose (61.00 cm). No consistent response was seen in Trinova genotype and reached the highest value at 0.2% dose (56.75 cm). Caramba genotype showed a slight increase and reached the peak at 0.6% dose (56.00 cm). Baqueno genotype showed a relatively high length at all doses and reached the highest values at 0.4% and 0.6% doses (59.25 cm). In terms of flag leaf width, the highest mean value was observed at 0.6% dose (5.97 cm). This was followed by 0.4% (5.92 cm) and 0.2% (5.74 cm) doses, while the control group had the lowest mean width (5.14 cm). These findings indicate that increasing zinc doses had a positive effect on flag leaf width, especially 0.6% dose, giving the best results in this parameter. According to the genotype × zinc sulfate dose interaction, it was observed that the width of Master genotype increased with zinc concentration and reached the maximum value (6.22 cm) at 0.6% dose. İlkadım genotype reached the highest width (6.87 cm) at 0.4% dose, indicating that it was more sensitive to medium levels of zinc application. A significant increase was observed in Trinova genotype and the highest width was obtained at 0.4% dose (6.02 cm). The Caramba genotype showed a limited response, reaching the highest width at 0.6% dose (5.65 cm). The Baqueno genotype showed a slight increase against doses, peaking at 0.6% (5.77 cm). When the averages were examined in terms of fresh forage yield, 0.4% (4.69 t/da) and 0.6% (4.72 t da^{-1}) doses had the highest yield values, followed by 0.2% (4.55 t da^{-1}). The control group showed the lowest average yield (4.34 t da^{-1}). This situation reveals that zinc application, especially at medium and high doses, increases fresh forage yield and 0.4% and 0.6% doses are ideal for this parameter. Master genotype showed a minimal response among all doses and provided a constant yield of 4.64 t da^{-1}. İlkadım genotype showed that it was positively affected by zinc application by reaching the highest yield at 0.6% dose (4.90 t da^{-1}). Trinova genotype slightly increased as the dose increased and reached the peak at 0.6% dose (4.59 t da^{-1}). The Caramba genotype showed a good response to increasing zinc doses by reaching the highest yield at 0.4% dose (4.80 t da^{-1}). Baqueno genotype also increased with the zinc dose and reached the highest value at 0.6% dose (4.77 t da^{-1}).

Finally, in the evaluation of hay yield, the highest mean value was obtained at 0.6% dose (1.47 t da^{-1}). This dose was followed by 0.4% (1.36 t da^{-1}) and 0.2% (1.32 t da^{-1}) doses, respectively, while the control group had the lowest hay yield (1.16 t da^{-1}). These data show that hay yield increased with increasing zinc doses and especially

the 0.6% dose provided the best yield. According to the genotype x zinc sulfate dose interaction, the Master genotype showed limited variation at different doses and reached the highest yield at 0.2% dose (1.39 t/da). The İlkadım genotype was positively affected by zinc doses, especially reaching the highest hay yield at 0.6% dose (1.71 t da^{-1}). The Trinova genotype reached its peak at 0.6% dose (1.37 t da^{-1}). While the Caramba genotype showed the highest hay yield at 0.6% dose (1.44 t/da), the Baqueno genotype also increased as the zinc dose increased and reached the highest yield at 0.6% dose (1.49 t da^{-1}) (Table 2.).

These evaluations detail the responses of each genotype to different zinc sulfate doses and point to potential yield strategies that can be optimized for each genotype according to zinc concentration.

Table 2. Mean comparison of agronomic traits in annual ryegrass genotypes as affected by foliar zinc sulfate

	Plant Height (cm)					**Leaf number per plant**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	91.79dh	93.68be	95.69bc	89.54fh	92.68 b	5.15h	5.93cf	5.93cf	6.15bd	5.79 bc
İlkadım	95.51bc	95.88b	101.16a	101.75a	98.57 a	5.59eh	6.48b	6.27bc	7.06a	6.35 a
Trinova	84.89ı	90.64eh	95.95b	90.54eh	90.50 c	5.18gh	5.83cf	5.96cf	6.21bc	5.80 bc
Caramba	88.23hı	91.98cg	100.80a	94.61bd	93.90 b	5.12h	5.50fh	5.67dg	5.97cf	5.56 c
Baqueno	88.87gh	93.13bf	93.62be	93.13bf	92.18 bc	5.56fh	5.59eh	6.08be	6.06be	5.82 b
Mean	89.85 c	93.06 b	97.44 a	93.91 b		5.32 c	5.87 b	5.98 b	6.29 a	
cv: 2.74 LSD_{var}: 1.82 LSD_{zinc}: 1.62 LSD_{int} : 3.63						cv: 0.71 LSD_{var}: 0.24 LSD_{zinc}: 0.22 LSD_{int} : 0.49				
	Flag Leaf Lenght (cm)					**Flag Leaf Width (cm)**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	50.50gh	55.50cf	56.25be	59.00ac	55.31 a	5.75cf	6.05bc	5.80be	6.22b	5.95 b
İlkadım	55.25df	52.25fh	59.25ab	61.00a	56.93 a	5.82bd	6.70a	6.87a	6.82a	6.55 a
Trinova	55.00ef	56.75be	55.50cf	54.00eg	55.31 a	4.20ı	5.32fh	6.02bc	5.37ch	5.23 d
Caramba	50.25h	52.00fh	54.50ef	56.00be	53.18 b	4.57ı	5.17h	5.27gh	5.65cg	5.16 d
Baqueno	52.00fh	56.75be	59.25ab	58.75ad	56.68 a	5.35eh	5.45dh	5.62ch	5.77bf	5.55 c
Mean	52.60 c	54.65 b	56.95 a	57.75 a		5.14 c	5.74 b	5.92 ab	5.97 a	
cv: 9.50 LSD_{var}: 1.78 LSD_{zinc}: 1.59 LSD_{int} : 3.56						cv: 12.06 LSD_{var}: 0.23 LSD_{zinc}: 0.20 LSD_{int} : 0.46				
	Fresh Forage Yield (t da^{-1})					**Hay Yield (t da^{-1})**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	4.45gı	4.64bf	4.64bf	4.63df	4.59 b	1.14ık	1.39bf	1.28fh	1.34dh	1.29 c
İlkadım	4.64df	4.69be	4.81ab	4.90a	4.76 a	1.28fh	1.44bd	1.48bc	1.71a	1.48 a
Trinova	4.09j	4.41hı	4.54fh	4.59ef	4.41 d	1.04k	1.26gı	1.22hj	1.37cg	1.26 d
Caramba	4.12j	4.43hı	4.80ab	4.72bd	4.53 c	1.11jk	1.22hj	1.40bf	1.44bd	1.29 c
Baqueno	4.39ı	4.58eg	4.69be	4.77ac	4.61 b	1.26gı	1.31eh	1.40be	1.49b	1.36 b
Mean	4.34 c	4.55 b	4.69 a	4.72 a		1.16 c	1.32 b	1.36 b	1.47 a	
cv: 2.06 LSD_{var}: 0.07 LSD_{zinc}: 0.06 LSD_{int} :0.01						cv: 6.39 LSD_{var}: 0.06 LSD_{zinc}: 0.05 LSD_{int} : 0.01				

The effects of foliar zinc sulfate application on the height of annual ryegrass (*Lolium multiflorum* Lam.) have garnered attention in agronomic research due to the significant role of zinc in plant growth and development.

Flag leaf traits, which are critical for crop yield, also benefit from foliar zinc applications. The flag leaf is essential for photosynthesis and grain filling in cereals, and its development can be enhanced by an adequate zinc supply. Research by Razzaq et al. indicates that foliar zinc application influences the mineral status of leaves, which in turn affects vegetative and reproductive growth, ultimately enhancing yield and quality (Razzaq et al., 2013). Moreover, the findings from Mantawy and Elhag emphasize that zinc sulfate application not only improves chlorophyll content but also enhances the overall canopy structure, which is vital for maximizing light interception and photosynthetic efficiency (Mantawy & Elhag, 2018).

Foliar application is recognized as an effective method for enhancing micronutrient uptake, particularly zinc, which is crucial for plant growth and development. Studies indicate that the application of zinc sulfate, both in soil and as a foliar spray, can lead to substantial increases in biomass and quality of forage crops. For instance, a meta-

analysis conducted by Iqbal et al. highlights the positive impact of foliar feeding of micronutrients, including zinc, on the productivity of forage crops across diverse climatic conditions. This analysis demonstrated that foliar application can lead to improved forage quality and yield, emphasizing the importance of micronutrient management in sustainable agriculture (Iqbal et al., 2019). Similarly, the research by Dhaliwal et al. supports the notion that biofortification through foliar application of zinc can enhance the nutritive potential of forage crops, thereby improving their overall yield and quality (Dhaliwal et al., 2022). Moreover, specific studies focusing on the effects of zinc sulfate on forage species such as oats and triticale have shown that the application of zinc can significantly improve morphological traits, dry matter yield, and crude protein content. For example, Sher et al. reported that different levels of soil-applied zinc sulfate, combined with appropriate harvesting stages, resulted in enhanced yield and quality of grass forages (Sher et al., 2022). This aligns with findings from a research, who noted that the combination of soil and foliar applications of zinc led to maximum plant height and leaf area index in forage oats, ultimately contributing to increased forage yield (Asif, 2024).

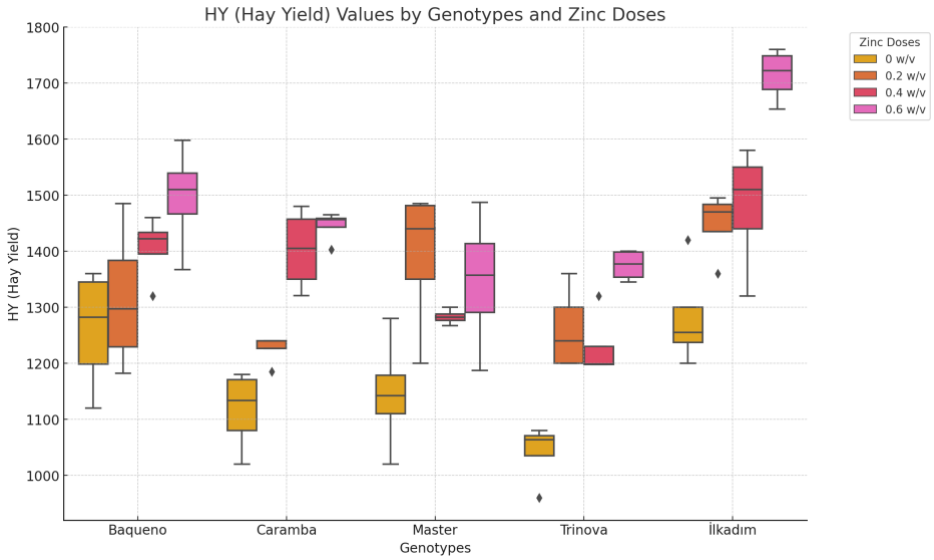

Figure 2. Boxplot of hay yield values by genotypes and zinc doses

The highest value in terms of leaf area was observed in İlkadım genotype at 0.4%, 0.6% and 0.2% doses (87.68 cm², 87.02 cm² and 87.02 cm²). This indicates that İlkadım responded positively to zinc application. On the other hand, the lowest leaf area value was obtained in Baqueno genotype at the control dose (74.60 cm²), indicating that this genotype was limited in leaf area development under zinc-free conditions. Master displayed the highest leaf area at 0.4% (87.06 cm²) and slightly decreased at 0.6% (86.77 cm²), suggesting an optimal response around 0.4% indicating a robust response to zinc. Trinova's leaf area showed minimal variation, with the highest at 0.2% (79.20 cm²), suggesting limited zinc sensitivity. Caramba had a slight increase at 0.6% (80.95 cm²), indicating a moderate response. Baqueno's leaf area also increased, with the highest value at 0.6% (80.73 cm²), suggesting a preference for higher doses. According to the mean values, the highest leaf area was obtained at 0.4% and 0.6% doses (82.41 cm² and 82.57 cm²). This result indicates that zinc sulfate promotes leaf development up to a certain level, but higher doses do not provide any additional gain. The control group had the lowest mean leaf area (78.97 cm²), indicating that zinc deficiency may limit leaf area.

With regard to chlorophyll content, the highest SPAD value was observed in the Caramba genotype at a 0.4% dose (57.42), indicating the potential of moderate zinc doses to enhance chlorophyll content. Conversely, the lowest SPAD value was noted in the Baqueno genotype at both the control and 0.2% doses (48.75), suggesting that zinc is a crucial factor for chlorophyll content increase. Master exhibited the highest SPAD value at 0.6% (56.05), indicating improved chlorophyll content at this dose. İlkadım's chlorophyll content increased gradually, peaking at 0.4% (55.80), reflecting a positive response to mid-range zinc doses. Trinova showed the highest SPAD at 0.6% (55.42), indicating zinc responsiveness. The highest mean SPAD value in terms of chlorophyll content was obtained at 0.4% dose (55.44), indicating that moderate zinc sulfate application provided the greatest increase in chlorophyll content. The lowest SPAD value was observed in the control group (52.26) and 0.2% (52.14), indicating that zinc-free medium had a negative effect on chlorophyll content.

The highest result in ADF values was observed in Baqueno genotype at 0.6% dose (32.17%). This indicates that high zinc concentrations increased the ADF ratio in Baqueno genotype. The lowest ADF value was obtained in Trinova genotype in the control group (28.41%), indicating that ADF content was lower under low zinc conditions. Master responded with moderate increases, peaking at 0.6% (30.84%), suggesting that higher zinc doses may enhance its ADF content. İlkadım exhibited the highest ADF at 0.4% (32.25%), indicating sensitivity

to mid-range doses. Caramba showed consistent increases, peaking at 0.6% (31.52%). The highest average ADF content was recorded at 0.6% (31.24%) and 0.4% (30.79%) doses. This result indicates that higher zinc doses tend to increase fiber content. The lowest mean ADF value was recorded in the control group (29.31%), suggesting that zinc deficiency may decrease the fiber content.

The highest value in NDF ratio was observed in İlkadım genotype at 0.6% dose (52.21%). This result reflects the positive response of this genotype to high zinc doses, while the lowest NDF value was obtained in İlkadım genotype at the control dose (47.26%). Zinc seems to be effective in increasing the NDF ratio. Master had a gradual increase in NDF, reaching a maximum at 0.6% (51.12%), suggesting sensitivity to higher doses. Trinova's NDF peaked at 0.6% (50.55%), similar to Master's, indicating responsiveness at high doses. Caramba's NDF was highest at 0.4% (52.66%), suggesting an optimal response at moderate doses. Baqueno's NDF increased steadily, peaking at 0.6% (51.69%). A similar trend was observed in NDF ratio; the highest average value was recorded at 0.4% dose (51.41%). In the control group, the NDF value remained at the lowest level (48.81%). This highlights the positive effect of zinc on plant fiber content and especially the role of medium zinc doses in increasing fiber content.

The genotype with the highest crude protein content was Baqueno, with a dose of 0.6%, which resulted in an impressive 16.28% protein yield. This finding suggests that zinc levels play a significant role in increasing protein content. Conversely, the lowest protein value was observed in the Ilkadim genotype at the control dose, indicating that protein content remained low in the presence of zinc deficiency. Master had the highest crude protein content at 0.6% (16.23%), suggesting a positive response to higher zinc doses. Trinova reached the highest protein content at 0.4% (16.00%), indicating mid-range dose sensitivity. Caramba's protein content was also highest at 0.4% (15.82%), showing an optimal response to moderate zinc The highest mean values in terms of crude protein content were observed at 0.4% (15.72%) and 0.6% (15.92%) doses. In zinc deficiency, crude protein content remained low and was recorded as 13.60% in the control group. These findings indicate that zinc application is effective in increasing protein synthesis and especially high doses can increase protein content.

The highest value in protein yield was obtained in İlkadım genotype at 0.6% dose (271.6 kg da^{-1}), indicating the positive effect of high zinc doses on protein yield. The lowest protein yield was recorded in Trinova genotype at the control dose (143.1 kg da^{-1}), indicating that protein yield was limited under zinc-free conditions. Master's protein yield increased significantly with zinc, peaking at 0.2% (223.9 kg da^{-1}) and then decreasing, indicating an optimal response at this lower dose. Caramba's protein yield was highest at 0.4% (222.1 kg da^{-1}), indicating sensitivity to moderate zinc. Baqueno's yield peaked at 0.6% (243.7 kg da^{-1}), showing a positive response to higher doses. Finally, the highest average value in terms of crude protein yield was obtained at 0.6% dose (234.9 kg da^{-1}). This result reveals that protein yield increased significantly with zinc application. The lowest protein yield was observed in the control group (159.0 kg da^{-1}), indicating that protein yield decreased in zinc-free environment.

The analyzed data reveal the relative feed value interactions between zinc sulfate doses and genotypes. Moreover, when the overall effect of each dose is evaluated over the mean values, the effect of zinc applications on RFV values in annual grass genotypes is clearly seen. The genotype İlkadım reached the highest RFV value at the control dose (131.1) and showed the highest value among all genotypes. However, this genotype decreased with zinc application and obtained one of the lowest values (113.8) at 0.4% dose. This suggests that İlkadım genotype may be sensitive to high zinc doses. Similarly, the Trinova genotype also responded positively to zinc treatments, and obtained a high RFV value (129.2), especially at the control dose. However, the RFV value in Trinova also decreased gradually with increasing zinc doses and the lowest value was observed at 0.6% dose (119.3). While Caramba genotype stood out with its high RFV value (125.3) especially at the control dose, the RFV value showed a downward trend with the effect of zinc applications. Caramba, with the lowest value observed at 0.4% dose (114.1), may have a less tolerant structure to zinc application. In Baqueno genotype, while the highest RFV value was observed at the control dose (122.0), this value decreased with zinc applications and the lowest value was observed at 0.6% dose (114.8). This indicates that Baqueno shows sensitivity to increasing zinc application.

When the mean RFV values were analyzed, the control group had the highest mean value (126.0), followed by 0.2% (122.1), 0.4% (117.5), and 0.6% (117.0) doses, respectively. This indicates that zinc sulfate applications caused a gradual decrease in RFV. In particular, 0.4% and 0.6% doses had the lowest mean RFV values, suggesting that high zinc doses may have a negative effect on RFV.

In the light of these data, it is seen that annual grass genotypes exhibited different sensitivities to zinc sulfate applications and RFV values generally decreased as zinc doses increased. While İlkadım and Trinova genotypes stood out with high RFV values at the control dose, zinc application caused a decrease in these values. In general, it can be said that low zinc doses had less negative effect on RFV, while high doses caused a decrease especially at 0.4% and 0.6% levels.

The impact of foliar zinc sulfate applications on the relative feed value (RFV) of forage crops has been extensively investigated in the scientific literature. The findings of these studies indicate that zinc applications have a significant effect on the nutritive value and digestibility of plants. In this study, zinc doses were observed to result in a general decrease in RFV, particularly at higher doses (0.4 % and 0.6 %). Similarly, the literature

discusses the effects of zinc on plant metabolism. Zinc is a vital microelement that plays a crucial role in supporting chlorophyll synthesis and enzyme activities in plants (Cakmak, 2008). However, excessive zinc applications can disrupt the biochemical balance of the plant, negatively affecting cell structure and consequently reducing plant digestibility (Alloway, 2008).

It has been demonstrated that zinc deficiency has a detrimental impact on plant yield and quality parameters. However, high doses of zinc applications can also result in toxicity, leading to adverse alterations in the nutrient composition of the plant (Broadley et al., 2012). The findings of this study indicate that the application of zinc can enhance plant productivity when utilized at optimal doses. However, excessive doses have been shown to reduce plant digestibility and nutritional value. Furthermore, some studies have indicated that zinc may enhance the synthesis of cell wall components and lignin, resulting in the hardening of plant tissue and a subsequent reduction in the digestibility of plant feeds (Marschner, 1995).

Table 3. Mean comparison of forage quality traits in annual ryegrass varieities as affected by foliar zinc sulfate

	Leaf area (cm^2)					**SPAD**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	79.46be	79.74be	87.06a	86.77a	83.26 b	51.92ef	50.35fg	52.70e	56.05ab	52.75 b
İlkadım	81.77b	87.02a	87.68a	87.02a	85.87 a	50.32fg	50.77f	55.80ab	55.00bc	52.97 b
Trinova	77.78cg	76.45eg	79.20bf	77.40dg	77.71 c	54.90bd	55.42b	56.05ab	55.42b	55.45 a
Caramba	81.23bc	75.54fg	79.77be	80.95bd	79.37 c	55.42b	55.42b	57.42a	53.22de	55.37 a
Baqueno	74.60g	78.47bf	78.36bf	80.73bd	78.04 c	48.75g	48.75g	55.25b	53.55bd	51.57 c
Mean	78.97 b	79.44 b	82.41 a	82.57 a		52.26 c	52.14 c	55.44 a	54.65 b	
cv: 3.22 LSD_{var}: 1.84 LSD_{zinc}: 1.65 LSD_{int} : 3.69						cv: 2.20 LSD_{var}: 0.84 LSD_{zinc}: 0.75 LSD_{int} : 1.67				
	ADF (%)					**NDF(%)**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	29.95bf	30.58ad	30.12bf	30.84ad	30.37 b	49.78fh	50.05eh	50.55dg	51.12bf	50.37 a
İlkadım	28.61ef	29.48cf	32.25a	30.81ad	30.29 b	47.26j	49.25gı	52.13ac	52.21ab	50.21 a
Trinova	28.41f	28.65ef	29.26df	30.88ad	29.30 c	48.06ıj	48.99hı	50.33dh	50.55dg	49.48 b
Caramba	29.29df	30.75ad	31.13ac	31.52ab	30.67 ab	49.15gı	50.16eh	52.66a	51.08cf	50.76 a
Baqueno	30.30be	31.61ab	31.21ac	32.17a	31.32 a	49.78fh	50.71cf	51.36ae	51.69ad	50.88 a
Mean	29.31 c	30.21 b	30.79 ab	31.24 a		48.81 c	49.83 b	51.41 a	51.33 a	
cv: 4.11 LSD_{var}: 0.88 LSD_{zinc}: 0.79 LSD_{int} : 1.77						cv: 2.03 LSD_{var}: 0.72 LSD_{zinc}: 0.64 LSD_{int} : 1.45				
	Crude Protein Content (%)					**Crude Protein Yield (kg da^{-1})**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	13.85hı	16.08ac	15.59bd	16.23ab	15.44 a	159.1jk	223.9bc	200.0dg	218.8de	200.4 bc
İlkadım	13.45ı	14.91eg	15.51ce	15.84ac	14.93 b	172.2ıj	215.9df	229.4bc	271.6a	222.3 a
Trinova	13.72ı	14.37gh	16.00ac	15.50ce	14.90 b	143.1k	181.3gı	196.6fh	213.0df	183.5 d
Caramba	13.39ı	14.45fh	15.82ac	15.73ac	14.85 b	149.3k	177.2hj	222.1c	227.4bc	194.0 c
Baqueno	13.59ı	15.08df	15.67ad	16.28a	15.16 ab	171.2ıj	198.7eg	220.2cd	243.7b	208.5 b
Mean	13.60 c	14.98 b	15.72 a	15.92 a		159.0 d	199.4 c	213.7 b	234.9 a	
cv: 2.74 LSD_{var}: 1.82 LSD_{zinc}: 1.62 LSD_{int} : 0.64						cv: 2.74 LSD_{var}: 18.2 LSD_{zinc}: 16.2 LSD_{int} : 20.84				

Table 4. Mean comparison of relative feed value in annual ryegrass genotypes as affected by foliar zinc sulfate

	Relative Feed Value				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**
Master	122.5cf	120.9cg	120.5dh	118.1fı	120.5 bc
İlkadım	131.1a	124.7be	113.8ı	115.6gı	121.3 b
Trinova	129.2a	126.4ac	122.1cf	119.3eı	124.3 a
Caramba	125.3bd	120.4dh	114.1ı	117.1fı	119.2 bc
Baqueno	122.0cf	117.8fı	117.0fı	114.8hı	117.9 c
Mean	126.0 a	122.1 b	117.5 c	117.0 c	

cv: 2.74 LSD_{var}: 1.82 LSD_{zinc}: 1.62 LSD_{int} : 5.67

Regression tables were constructed to elucidate the degree and direction of the relationship between the dependent and independent variables utilized in the study. These tables serve as a valuable tool for elucidating the impact of genotypes and zinc doses on plant growth parameters. In this study, regression coefficients demonstrate the positive or negative influence of specific zinc doses on plant productivity The variation of the values does not reveal a linear increase. This is due to the low R^2 values (SPAD: 0.18; leaf area: 0.11; hay yield: 0.41; crude protein yield: 0.61). The inclusion of many values may be difficult for the description of the modeling. However, these tables are also provided to explain the interactions.

These findings offer crucial insights into the effects of zinc applications on crop yield and quality (Figure 3.).

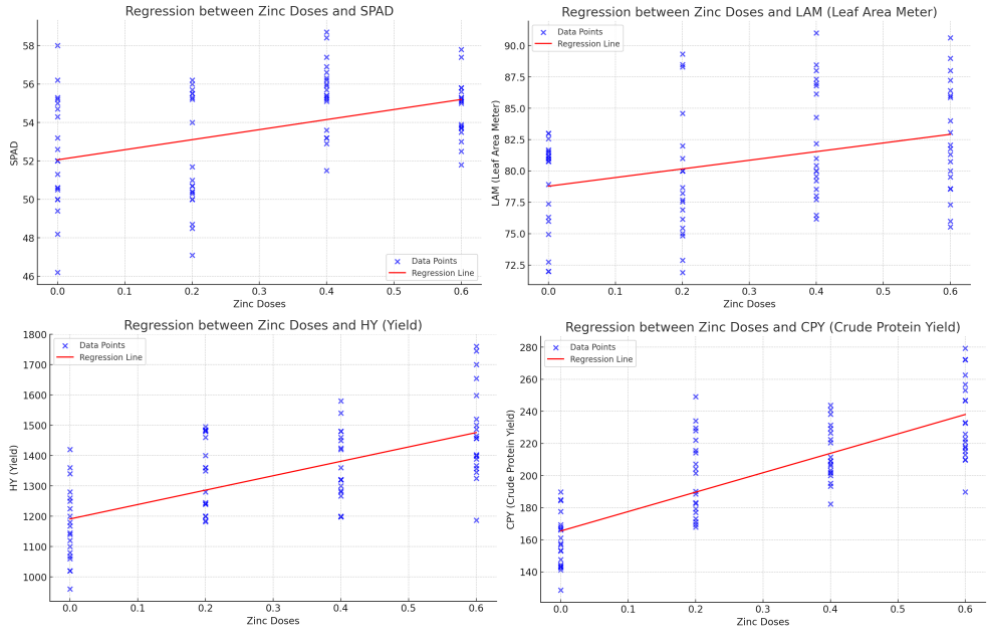

Figure 3. Regression analysis of the relationship between zinc doses and SPAD, leaf area, hay yield and crude protein yield values

Correlation analysis examines the linear relationship between the variables used in the study and reveals the interactions of these variables with each other. Correlation coefficients indicate the direction and strength of the relationship between variables. Positive correlation coefficients indicate that two variables increase or decrease together, while negative correlation coefficients indicate that one variable increases while the other decreases. Parameters with high positive correlation values in the analysis indicate that these variables have a strong linear relationship with each other. For example, high positive correlations between plant height and fresh forage yield or crude protein ratio and crude protein yield indicate that these parameters support each other. In contrast, negative correlations may indicate an inverse relationship between certain parameters The strongest positive interaction was observed between hay yield and crude protein yield with 0.95. The strongest negative interaction was found between NDF and RFV with 0.97 (Figure 4.).

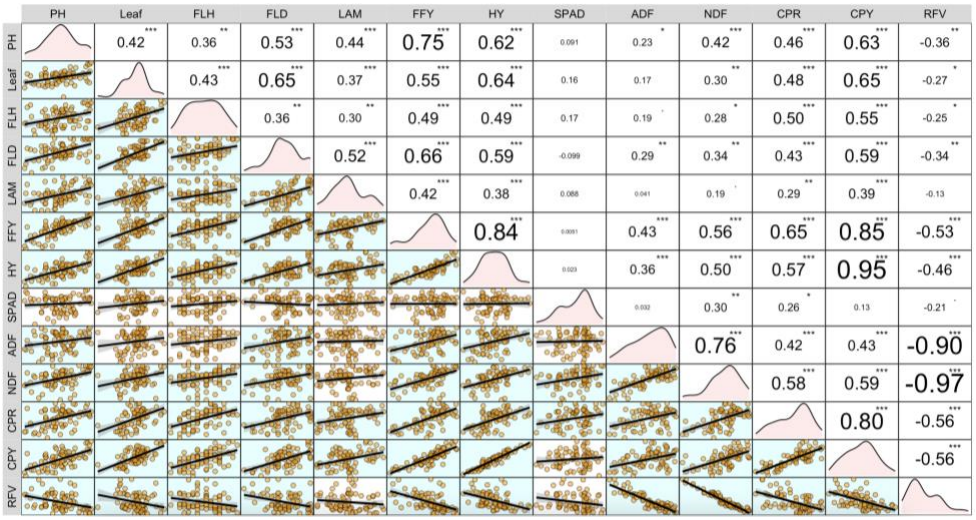

Figure 4. Pearson's correlation analysis of variables in the experiment (PH: Plant height; FLH: Flag Leaf Height; FLD: Flag leaf width; LAM: Leaf area per plant; FFY: Fresh forage yield; HY: Hay yield; CPR: Crude protein content; CPY: Crude protein yield; RFV: Relative feed value)

CONCLUSION

The cultivation of annual ryegrass is increasing due to its superior characteristics in terms of yield and quality, as well as the positive impact it has on livestock. While there are no specific requirements for cultivating this species, soils lacking sufficient macro- and micronutrients can impede the attainment of optimal yields and quality. Zinc is of particular significance in this context. The objective of this study was to investigate the impact of zinc sulfate application on the agronomic and quality characteristics of zinc-deficient soils. In the study in which different genotypes were tested, it was observed that the genotypes exhibited disparate values concerning their genetic characteristics. Nevertheless, in general, there were notable increases in yield, fiber properties, and crude protein yield with the application of zinc. Among the genotypes, İlkadım and Baqueno exhibited the highest yield and quality values in these increases. As the increase in zinc doses was not significantly different between 0.4% and 0.6%, the application of a 0.4% zinc sulfate dose was deemed sufficient for this experiment. However, it was determined that the optimal dose and genotype can be selected on an economic basis by considering cost calculations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Thre are not any conflict of interest.

Author contribution

EK design, experiment and writing. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Household pesticide use: attitudes, behaviors, and health risks

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DergiPark AKADEMİK

Abstract

The main objective of the scientific study was to determine the level of use and attitudes toward the use of pesticides designed for domestic use. Pesticides, intentionally released toxic substances, have the potential to cause adverse health effects even at very low exposure levels, including hormonal disorders, asthma, allergies, and cancer. The rate of pesticide usage and attitudes toward pesticides in the home were measured through an online questionnaire that was completed by a total of 250 respondents. Based on the data collected, we found that the majority of respondents use pesticides in their homes to control insects. The questions in the questionnaire were focused on the respondents' practices in handling, application, storage, disposal, and purchasing of pesticides. Exposure to pesticides can result in mild symptoms, such as vomiting, through to more severe consequences like cancer. Despite the risks associated with pesticide use, regulatory measures are in place; however, misuse often stems from improper storage and disposal practices. We found that gender did not influence safe pesticide handling behavior, although women exhibited more responsible behaviour in purchasing, storing, and disposing of pesticides. Compared to men, women had a higher overall mean score for attitude towards pesticides and thus approached pesticides more responsibly than men. We examined differences in pesticide handling behaviour between urban and rural residents. Based on the data collected, we found that attitudes towards pesticides of urban and rural dwellers are not different. Through the statistical analysis, we concluded that respondents' attitudes towards pesticides influence safe behaviour in purchasing, handling, storage, and disposal of pesticides. The more positive the respondents' attitude toward pesticides, the more responsible their attitude towards pesticides.

Keywords: Pesticides, Attitude, Behaviour, Household, Safety, Enviroment

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INTRODUCTION

Definition of Key Terms

Pesticides are substances or agents used to affect the basic life processes in an organism (Frankovska et al., 2010). These substances are one of the few toxic substances that are intentionally released into the environment by human activities (Kim, Kamir, Jahan, 2017). Worldwide, nearly 3 billion kilograms of pesticides are used annually for various purposes (Sharma et al., 2020). Of the 1,361 pesticide-active substances registered in the European Union, 489 (35.9%) active substances are authorized for plant protection. Attitudes toward pesticides are shaped by a variety of factors, including access to information, education level, and participation in training courses on pesticides. These factors significantly influence how people follow the pictograms and instructions on pesticide products (Bagheri et al., 2021). Education and awareness play a critical role in promoting responsible pesticide use, as individuals with better access to information are more likely to adhere to safety guidelines. The concept of "attitude" appeared in psychology as early as the 18th century and was defined in 1860 as a state of the internal environment ready to act. The term "attitude" was first mentioned in the sense of individual action and consciousness at work (Cacioppo et al., 1994; Mónus, 2022). Based on its structure, attitudes are divided into a cognitive component (knowledge about the object), an affective component (emotions and feelings towards the object), and a conative component (behavior towards the object) (Ostrom, 1989). The development and formation

of attitudes are largely influenced by the amount and quality of information that individuals possess. There are many ways to influence attitudes, with education being the foundation (Horan et al., 2022). The most reliable method for measuring attitudes is considered to be the method of attitudinal scales, which are bipolar and composed of statements and their intensity that the respondent selects (Tajfel, 1982). According to Stern (2000), environmental values and attitudes are fundamental for developing pro-environmental behavior. People strive to align what they believe is right with what they actually do. When environmental values and attitudes are confronted with personal responsibility, they often lead to a sense of moral obligation and responsible action (Kanzler et al., 2022). This connection between values and behavior is crucial in fostering a responsible approach toward pesticide use. Each pesticide product label contains a safety warning that lists the requirements for protective measures. The user is responsible for reading and following the instructions on the product labels (Hansen, Walker, & LEGAULT, 2015). Proper use of gloves and other protective equipment is as important as their selection (KWON, Campbell, & Zirwas, 2006). Teachers play a pivotal role in shaping the attitudes of students. Research by Ulug et al. (2011) demonstrated that positive attitudes held by teachers can influence the personalities and future behaviors of their students. This shows that education not only imparts knowledge but also molds attitudes, which is particularly relevant when it comes to topics like pesticide use and environmental conservation. Palmer et al. (1993) found that the most significant predictor of pro-environmental behavior was individuals' childhood experiences with nature, such as time spent in gardens or other natural settings. Hinds & Sparks (2008) discovered that students raised in rural areas tend to have more positive attitudes towards the environment compared to those raised in urban areas, suggesting that direct exposure to nature plays an important role in shaping environmental attitudes. Gender differences in attitudes toward the environment have also been extensively studied. Many authors have found that girls tend to have better environmental attitudes than boys (Bulut, 2019; Collado et al., 2017). This indicates that gender may also influence how individuals perceive and engage with environmental issues, including the responsible use of pesticides.

Current State of Literature

Even very low levels of exposure to pesticides can have adverse effects on human health, including hormonal disorders, asthma, allergies, and cancer (Kım, Kamır, & Jahan, 2017). Pesticide poisoning is most dangerous when the pesticide enters the human body through the oral route. Oral exposure to pesticides most commonly occurs accidentally due to inattention (spilling pesticide into an unlabeled bottle, improper storage, children's access to pesticides) or due to intentional suicidal reasons (Gılden et al., 2010). Despite sufficient regulation and stringent pesticide legislation, it is estimated that three million cases of pesticide poisoning occur worldwide each year, resulting in more than 250,000 deaths (World Health Organization, 2006). Exposure to pesticides has been linked to various diseases, from mild symptoms such as vomiting to serious conditions like cancer or death (Thundıyıl et al., 2008; Alavanja, Hoppın, Kamel, 2004). Many authors have addressed the proper use of pesticides in households. Since the home environment is generally considered the most pesticide-treated indoor environment, improper use of pesticides poses significant risks to both humans and domestic animals (NALWANGA and SSEMPEBWA, 2011). Studies indicate that men and women behave differently in pesticide use. More men disposed of pesticide containers in an environmentally friendly way, but fewer men applied self-protective measures when using pesticides (Wang et al., 2017, Jin et al., 2015; FAO, 2011). Attitudes toward pesticides are crucial in determining responsible pesticide use. Currently, the term attitude is most often used to refer to a relatively general and sustained evaluation of an object or concept, ranging from the positive to the negative range (Fabrıgar and Wegener, 2010). Attitudes can include a cognitive (rational), emotional (affective), or conative component (Bızer, Barden, & Petty, 2006). Research shows that friends and relatives often influence individuals' choices regarding pesticides. Gray et al. (2005) reported that 53% of respondents relied on friends and family members for insecticide selection, while over 33% used radio and television advertisements. Other sources of information, such as point-of-sale and retail outlets, accounted for 48%. Azratul-Hızayuz et al. (2021) conducted research in Selangor, Malaysia, and found that up to 90% of the respondents read the manufacturer's instructions before using pesticides. In Nigeria, Adje and Aremu (2020) reported that 79% of respondents read product labels before using pesticides. However, Nalwalnga and Ssempebwa (2011) found that only 48% of respondents read the manufacturer's instructions before applying pesticides in their homes. Proper storage and disposal are also crucial: 88% of respondents stored excess pesticides, and 10% disposed of them improperly. Only 2% recycled pesticide packaging (Grey, Nıeuwenhuıjsen, Goldıng, 2005). Similar findings were reported by Adje and Aremu (2020), who found that most empty pesticide containers were discarded or incinerated, with only 5% being recycled. Finally, pesticide handling practices such as washing hands after pesticide use (64%) or washing used clothing (4%) remain insufficient (Azratul-Hızayuz et al., 2021; Dje and Aremu, 2020). These practices highlight the need for greater awareness and education on safe pesticide use among end-users.

MATERIALS AND METHODS

Respondents

The work was carried out in the Slovak Republic, while the selection of respondents was random. The research sample consisted of 250 respondents. We had to exclude two persons from the age category 65 years and older

from the analysis because of the low representation of this age category (Table 1). Respondents were selected randomly; the only condition for participation in the research was that respondents were at least 18 years old. The research was conducted using an online questionnaire, which was posted on the social network Facebook from 01 November 2021 to 10 December 2021. These months were deliberately chosen after the summer months, when people most often use pesticides, especially against unwanted vegetation and insects.

Research Tool

A Likert scale questionnaire is most commonly used to measure the attitudes of participating respondents (Gáborová, Porubčanová, 2016). The questionnaire was anonymous and intended for adults. The questionnaire contained 15 questions and 9 statements. The research instrument was divided into two parts, with the first part of the questionnaire containing 15 questions with one correct answer choice and one multiple choice question. The second part of the questionnaire included nine statements with a Likert scale. Respondents used the statements: strongly agree, agree, don't know, disagree, and strongly disagree to express their level of agreement or disagreement with a given statement about pesticides.

Data Analysis

We then scaled the collected data and calculated the average attitude in the three question areas: pesticide purchasing, handling, and application, pesticide storage, and pesticide disposal. We scored the results by assigning a value of 5 to statements of strongly agree and a value of 1 to statements of strongly disagree. For negative statements, we rescored in reverse. After, we calculated the average score. The data collected were recorded into a table (Microsoft Excel), which serves as a basis for this work. The statistically processed data were subjected to an analysis in the Statistica Programme, version 12.

Table 1. Division of respondents.

Gender		Age					Residence	
Men	Women	18-25	26-35	36-45	46-55	56-65	Village	Town
72	176	170	27	34	12	5	167	81

RESULTS

The results section was divided into three categories, according to the focus of the questions on purchasing, handling and application, and storage and disposal of pesticides. This division corresponds to the three question areas from the questionnaire.

Purchase of pesticides

In the section on pesticide purchase respondents answered questions 6, 7, and 8 which were focused on motivation to use the pesticide and reading the product label. Most respondents, 33%, reported that they purchase pesticides based on recommendations from friends and family. Further, more than 28% of respondents reported that they purchase pesticides based on the information on product labels, and more than 27% reported purchasing pesticides based on retailer recommendations. Only 6% of respondents buy pesticides according to the pictures on the product packaging without further information about the product. Only 4% of respondents buy pesticides based on advertisements for home use. 56% of respondents follow the information and instructions given on the product labels. The instructions and information on the product labels are followed by 39% of respondents only if they are using the product for the first time. Around 5% of respondents reported that they do not follow the labels or do not read the instructions on the product labels.

Manipulation with pesticides

Another area of questions in the questionnaire was related to pesticide handling. This area was covered by questions 9 and 10, which dealt with the use of personal protective equipment. Questions 11 and 12 focused on safety precautions after pesticide application, such as hand washing. Protective gloves are not used when handling pesticides by 25% of respondents, protective clothing is not used by up to 71% of respondents, 20% of respondents stated that they only use protective clothing if it is stated on the product label, and 3% of respondents only use protective clothing if they are using the pesticide for the first time. After pesticide application, 92% of respondents always wash their hands, while up to 8% of respondents do not wash their hands after pesticide application. Some respondents reported that they do not wash their hands if they use protective gloves or if it is not stated on the product packaging. Ventilating the room where the pesticide is applied is always done by up to 84% of respondents, with 12% doing so only if it is stated on the product label and 4% ventilating the room only if there is a pet, a small child nearby or if it is the first time they have used the pesticide.

Storage and disposal of pesticides

The last area of questions was aimed at the storage and disposal of pesticides. Questions 13, 14, and 15 deal with the topic of this section. A storage location in the home that is out of reach of children and pets is used by 81% of respondents, 14% of respondents use a cellar to store pesticides and 5% of respondents use a shed in the garden. None of the respondents store pesticides near medicines and food. More than 79% of respondents store pesticides in their original labeled containers, according to the instructions on the product labels. The second most

common way to store pesticides is in open original containers with labels. This option was chosen by 18% of respondents. Two percent of respondents indicated that they store pesticides in new containers with the original label or store pesticides in original containers without a label with a self-label or without label. Approximately 50% of respondents indicated that they dispose of packaging according to the instructions on the product and 24% of respondents dispose of the packaging in unsorted waste, and 24% of respondents indicated that they dispose of the packaging in sorted waste.

The frequency of usage of pesticides and its influence

When asked how often respondents use pesticides, more than 55% answered that they use pesticides once a year. More than 34% of the respondents use pesticides once a month and only about 6% of the respondents use pesticides once a week. The remaining respondents used pesticides at a lower frequency. The next question was used to find out the main reason for pesticide use by the respondents. Respondents could choose several options. More than 81% of respondents use pesticides to control insects. Pesticides against rodents are used by 23% of the respondents and more than 20% of the respondents stated that the main reason for using pesticides is fungi. We have shown that the frequency of pesticide use does not have a significant effect (p=0.407) on the rate of pesticide use method (Figure 1).

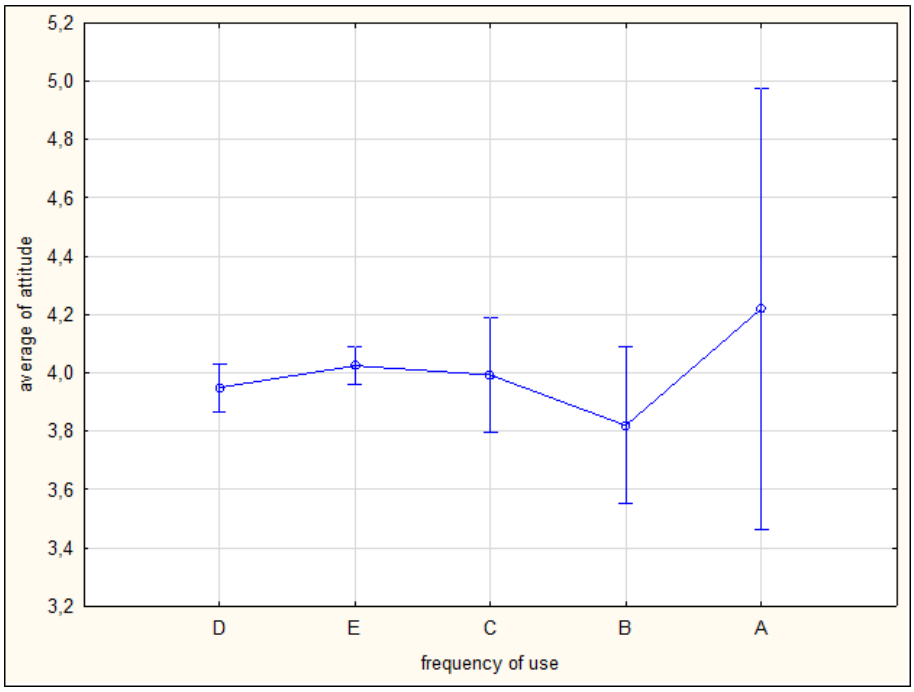

Figure 1. Impact of frequency of use on the rate and pattern of pesticide use
A - every day, B - three times a week, C - once a week, D - once a month, E - once a year

We investigated how the frequency of pesticide use influences responsible pesticide purchasing behaviour. We found that respondents who use pesticides the most frequently - three times a week are the least responsible when buying pesticide products. Respondents who use pesticides once a year behave the most responsibly and safely when purchasing pesticides. Frequency of pesticide use has a significant effect (p=0.005) on pesticide purchasing, the more frequently respondents used pesticides, the less responsible they were in purchasing pesticides (Figure 2).

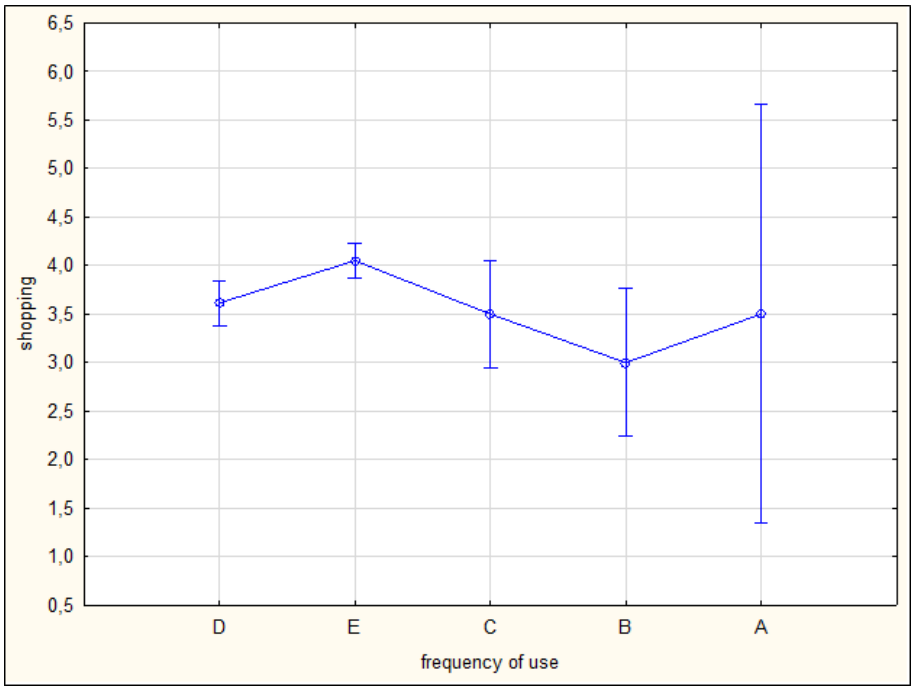

Figure 2. Impact of frequency of use on pesticide purchasing
A - every day, B - three times a week, C - once a week, D - once a month, E - once a year

Further, we found a statistically significant difference (p=0.051) for respondents who used pesticides most frequently and were the most responsible in handling and applying pesticides. Respondents that use pesticides once a year (E) had the second highest score. Respondents that use pesticides once a week (C) had the lowest rate of appropriate pesticide handling (Figure 3). We did not observe an effect of frequency of pesticide use on pesticide storage and disposal (p=0.306).

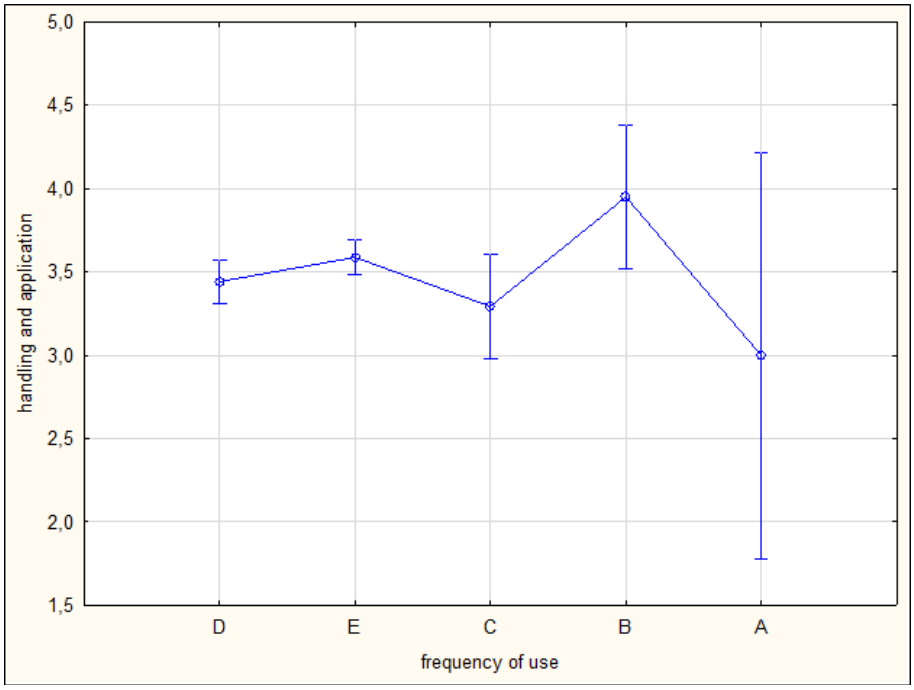

Figure 3**.** Impact of frequency of use on handling and application of pesticides

Influence of gender

In another research investigation, we looked at the impact of gender on pesticide handling, pesticide purchasing, and pesticide application. We did not find a statistically significant difference, but the p-value was at the borderline of provability (p=0.075). Women have higher mean scores for attitude towards pesticides compared

to men, thus showing an indication that women are more responsible in their approach towards pesticides (Figure 4).

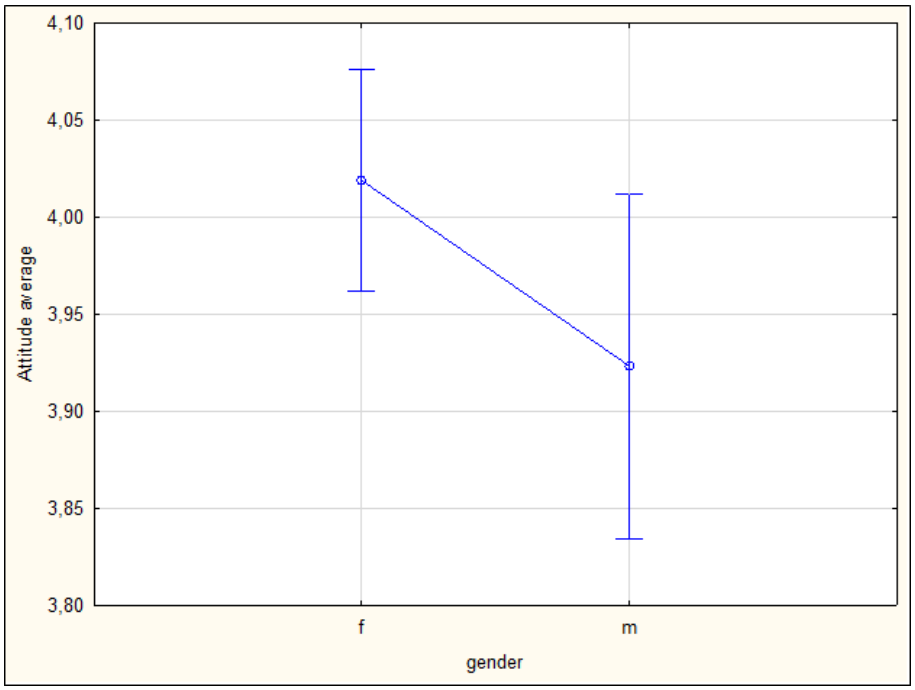

Figure 4. Influence of gender on the rate and pattern of pesticide use

We further demonstrated that gender is statistically significant (p=0.035) in pesticide purchasing. Women take a more responsible approach to pesticide shopping compared to men (Figure 5). Gender had no statistically significant effect on the handling and application of pesticides (p=0.909). Similarly, we showed no statistically significant difference in pesticide storage, although women scored better than men (p=0.136).

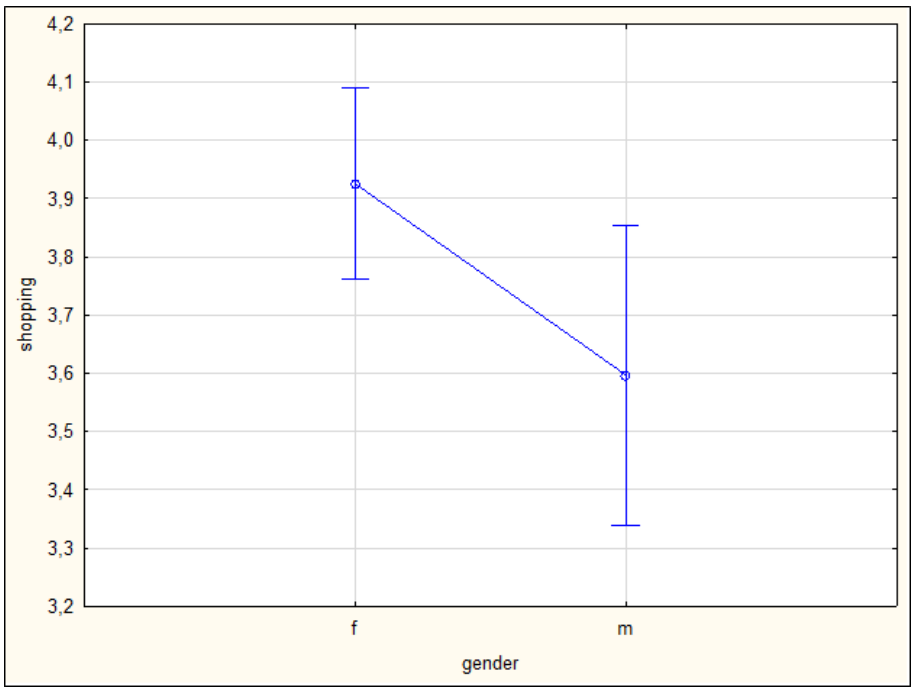

Figure 5**.** The influence of gender on purchasing of pesticides

Influence of residence

In the statistical evaluation of the data, we found that respondents from the city had a higher mean score on attitudes towards pesticides, but on the other hand, we did not find a statistically significant difference between people living in the city and those living in the countryside (p=0.648). Similarly, we did not show a significant

difference in pesticide-buying behavior between urban and rural dwellers (p=0.979). Further, we did not show any difference in pesticide handling and application behavior between urban and rural dwellers (p=0.979). The storage and disposal of pesticides for home use are also not influenced by the respondent's place of residence (p=0.267).

Correlations between respondents' behaviour and attitude

We demonstrated that the more positive the respondent's attitude towards the safe handling of pesticides, the more responsible the respondents were in handling and applying pesticides (p=0.0001). We also demonstrated that the more positive the respondent's attitude towards pesticides, the more responsible the respondent was in storing and disposing of pesticides (p=0.0001). The higher the mean score of the respondents' attitude towards pesticides, the more responsible they were in purchasing pesticides (p=0.002). It can be argued that attitudes towards pesticides are positively correlated in all the areas we looked at.

DISCUSSION

The aim of the present work was to find out the extent and pattern of pesticide use in households and the effect of attitude on pesticide use. We focused on the practices that people use in purchasing, storing, and disposing of pesticides and in handling and applying pesticides in the home. Most respondents use pesticides in their homes to control insects. When shopping for pesticides, 33% of respondents buy the products based on recommendations from friends and family. Further, more than 28% of respondents reported buying pesticides based on information on product labels and more than 27% reported buying pesticides based on retailer recommendations. GREY et al. (2005), in their study on household pesticide use and disposal, reported that 53% of respondents relied on friends and family for insecticide selection, followed by 48% of respondents who cited point-of-sale as an important source of information. We also found that the majority of respondents, over 56%, always read and follow the information and instructions on product labels when using pesticides. Azratul- Hızayuz et al. (2021), based on research conducted abroad, reported in their study that up to 90% of the respondents surveyed read the instructions given on product labels before using a pesticide. Adje and Aremu (2020) also obtained similar results. Based on the data obtained from our research, we conclude that the majority of the respondents, 81% store pesticides in a safe place designated for that purpose, and more than 79% of the respondents store pesticides in the original labeled containers according to the instructions given on the product labels. Similar conclusions were reached in the work of (Azratul-Hızayuz et al., 2021 and Adje and Aremu, 2020). We confirmed hypothesis 1, with our research results indicating that the higher the respondents' scores in their attitudes towards safe pesticide handling, the more responsible their practices in handling and applying pesticides were. Our findings are supported by the works of (Azratul-Hızayuz et al., 2021; Gholamı et al., 2018). We did not confirm hypothesis 2, which focused on women behaving more responsibly in pesticide use than men. Wang et al. (2017) found that there is a gender gap with respect to knowledge of pesticide impacts and pesticide use practices. Dunlap et al. (1992) report that women are significantly more concerned about pesticide safety. We have not demonstrated the influence of residence in purchasing, disposing of, or using pesticides. Similar results were also reached in the work of Coppın, Eısenhauer, and Krannich, 2003.

CONCLUSION

The results of this study showed that respondents' attitudes toward pesticides are correlated with their purchasing, disposal, or user behavior. Among the factors studied, we did not show the influence of gender and place of residence, in more responsible pesticide behavior. We did demonstrate an effect of frequency of pesticide use, with respondents who use pesticides more frequently behaving less responsibly. It can be concluded that the problem of pesticides plays an important role in environmental pollution and human health protection. By improving peoples' attitudes, we can help to solve this problem in society.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

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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Evaluation of the efficacy and residual activity of Chlorantraniliprole 600 g/L SC against fall armyworm *Spodoptera frugiperda* (J.E. Smith,1797) (Lepidoptera: Noctuidae) on Corn (*Zea mays* Linnaus, 1753)

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DergiPark AKADEMİK

Abstract

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith,1797), (Lepidoptera: Noctuidae) is a polyphagous pest that has a worldwide distribution. It is an invasive species and can cause significant damage to corn. In this study, the effectiveness of four doses (2, 3, 4, and 5 ml/da) of Chlorantraniliprole 600 g/l against *S. frugiperda* was first investigated. Then, the residue amount of 5ml/da dose of Chlorantraniliprole 600 g/l was determined. Efficacy trials were conducted in arable fields in Adana and Şanlıurfa provinces from August to September 2023 according to the randomized block design with five characters (four doses of the trial insecticide and control) and four replications. Residue experiments were carried out in Adana and Şanlıurfa provinces. When the seventh day counts were evaluated, mortality from the 2 ml/da dose was between 39.67 and 38.84%; mortality from the 3 ml/da dose was between 56.68 and 57.53%; mortality from the 4 ml/da dose was between 75.05 and 77.22%; and mortality from the 5 ml/da dose was between 93.96 and 93.86%. As a result of the sampling, the residue amount of Chlorantraniliprole in corn was found to be below the residue limit determined in Türkiye and Europe. It was concluded that a dose of 5 ml/da of Chlorantraniliprole 600 g/l can be used as an effective dose against *S. frugiperda*.

Keywords: Chlorantraniliprole, Corn, Residue, *Spodoptera frugiperda*

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INTRODUCTION

Corn is one of the most important cereal crops consumed in the world. In 2022, 1.129 million tons of corn were produced in 206 million hectares of planting area globally. In Türkiye, 8.5 million tons of corn were produced in 9.1 million da of planting area (Anonymous, 2023a). *Spodoptera frugiperda* (J.E. Smith, 1797), (Lepidoptera: Noctuidae) is a polyphagous pest and causes damage to many crops, including corn, rice, sorghum, and cotton (Montezano et al., 2018). The pest, which was first seen in cornfields in Adana province in Türkiye in 2022, has a wide distribution around the world (Wang et al., 2020; Pehlivan and Atakan, 2022; Mohamed et al., 2022). The larvae of the pest damage the leaves of the plants and can also cut the growth centers of the plants and cause them to dry out. Damage may increase depending on the larvae density (Anonymous, 2019). Chlorantraniliprole is known as a systemic insecticide with broad spectrum activity against Lepidoptera pests. (Adams et al., 2016). It leads to paralysis and death by activating the insect's ryanodine receptors and causing the internal calcium stores to be lost (Cordova et al., 2006). For the harvested products to be consumed healthily and safely, attention needs to be paid to the acceptable residue rate. The aim of this study was to evaluate the effectiveness of four doses (2, 3, 4, and 5 ml) of Chlorantraniliprole 600 g/l SC against *S. frugiperda*. In addition, the residue amount of the active substance at a dose of 5 ml/da was determined.

MATERIALS AND METHODS

Evaluation of the Effectiveness of Chlorantraniliprole (600 g/L)

The trial was conducted in cornfields in Adana (37°08'32.3"N 35°18'49.1"E) and Şanlıurfa (36°55'39.7"N 38°29'53.5"E) provinces in the period from August to September 2023 using a randomized block design with five characters (four doses of the insecticide and control) and four replications. The provinces selected are located in different geographical regions. Since there is no plant protection product with similar properties to Chlorantraniliprole, such as active substance and percentage, formulation, effect and application method, licensed as a comparator insecticide in the study, it was tested comparatively with the control. During the trial, it was determined that the plants were 30-35 cm tall, and that phenologically the plants generally had six leaves (BBCH 16). P 1551 hybrid varieties are used in Adana, while P0900 hybrid varieties are used in Şanlıurfa in arable fields (Seed date: 07.07.2024). In the examination carried out in the field in the direction of diagonals before the trial, fall armyworm larvae were searched for in the plant residues and weeds around the roots of randomly selected plants. As a result of this examination, it was determined that the larvae were scattered on the plants and there were more than two larvae (one-to-three stages) per plant. The plot size in the experiment was determined as 105 m² (10 plant rows x 15 m). Surface spraying was carried out in Adana on 05.09.2023 and in Şanlıurfa on 12.10.2023. The spray was applied to all plants in the plot in such a way that it would be distributed homogeneously. During the spraying, care was taken to wet all the green parts of the plants from top to bottom and also the weeds and plant residues around the plant. Counts were made on five different points in the inner part of the plot, after leaving sufficient safety strips at the beginning, sides and ends to reduce the edge effects of the plot, and on five plants located next to each other in the same row, for a total of 25 plants. During the count, all above-ground parts of the plant and the plant residues and weeds around it were examined and the live larvae found were counted and recorded. Counts were made before spraying and three, seven and 14 days after spraying. In the analysis, the results of the counting results were determined by applying the Henderson-Tilton formula to live larvae to determine the percentage effects of the insecticide. Variance analysis and the Duncan test were applied to the angle values of the percentage effects of the doses. No other pesticide was applied in the experimental area during the study.

Evaluation of the Residue of Chlorantraniliprole (600 g/L)

The study was conducted in arable fields in Adana and Şanlıurfa. The study included two characters, namely the recommended 5 ml/da dose of insecticide and the control. Each plot in the experiments was 100 m^2 and three sprayings were performed at one-week intervals. To ensure homogeneous distribution of the pesticide to the plants in the plot, each row was entered and care was taken to spray every part of the plant thoroughly. Since the period between the last spraying and harvest (PHI) was predicted as 14 days by the company, six samples were taken from corn according to the "Standard Test Method for Testing Residues of Plant Protection Products on Plants or Plant Products". These samples were taken on day 0, and the first, fifth, 10th, 14th and 21st days after spraying. Samples were taken from the sprayed plot and the control plot on the same day. To obtain sufficient amount of corn grain sample, more than 12 plants were cut from different parts of the inner part of the plot and 12 cobs were randomly taken from these plants (Anonymous, 2023b). Samples for each character were labeled in separate containers and stored in an ice box (-20 °C). After the sampling process was completed, all samples were delivered to BİLÇEV Private Food Control Laboratory for residue analysis by preserving the cold chain feature. The QUECHERS extraction method was applied in residue analysis and the extracted products were analyzed with LC-MSMS device.

RESULTS AND DISCUSSION

The effects of Chlorantraniliprole 600 g/l on live larvae (%) are given in Table 1.

When Table 1 is examined, it is seen that the greatest effect of Chlorantraniliprole 600 g/l was seven days after spraying in both regions. The greatest effect was found at a dose of 5 ml/da in both regions. The effect on live larvae was 93.96% in Adana and 93.86% in Şanlıurfa, and these results are statistically significant. Chlorantraniliprole is a systemic insecticide and is highly effective against lepidoptera (Adams et al., 2016; Moustafa et al., 2021). It is known that this active substance can activate the ryanodine receptors of the insect, causing paralysis and death. Moreover, it has been reported in studies that it has a very strong effect on *S. frugiperda* (Li et al., 2021; Meghana et al., 2023; Song et al., 2023). The results obtained in our study support the results of these studies.

The residue amounts in the residue trial conducted in cornfields in Adana and Şanlıurfa provinces are given in Table 2.

Table 1. Mortality effect of Chlorantraniliprole 600 g/l on *Spodoptera frugiperda* larvae in Adana and Şanlıurfa provinces (%)*

Characters	Adana (larvae%)				Şanlıurfa (larvae%)			
	T+0 (Mean larvae)	T+3	T+7	T+14	T+0 (Mean larvae)	T+3	T+7	T+14
Chlorantraniliprole 600 g/l (2 ml/da)	90.50a	38.05d	39.67d	34.59d	83.00a	37.95d	38.84d	34.62d
Chlorantraniliprole 600 g/l (3 ml/da)	92.00a	55.40c	56.68c	50.84c	84.00a	56.26c	57.53c	51.86c
Chlorantraniliprole 600 g/l (4 ml/da)	91.50a	73.97b	75.05b	66.60b	83.00a	75.14b	77.22b	66.35b
Chlorantraniliprole 600 g/l (5ml /da)	92.50a	92.41a	93.96a	83.62a	82.75a	92.93a	93.86a	85.03a
Control	91.25a				84.00a			

*Means followed by different lowercase letters are significantly different (P<0.05) within a column for the first

Table 2. Residue amounts in the residue trial conducted in cornfields in Adana and Şanlıurfa provinces

Parcels	Sampling day(T:treatment)	Adana Chlorantraniliprole (mg/kg)	Şanlıurfa Chlorantraniliprole (mg/kg)
Control	T+0	<0,010	<0,010
Application	T+0	<0,010	<0,010
Control	T+1	<0,010	<0,010
Application	T+1	<0,010	<0,010
Control	T+5	<0,010	<0,010
Application	T+5	<0,010	<0,010
Control	T+10	<0,010	<0,010
Application	T+10	<0,010	<0,010
Control	T+14	<0,010	<0,010
Application	T+14	<0,010	<0,010
Control	T+21	<0,010	<0,010
Application	T+21	<0,010	<0,010

Considering all the sampling, the residue amounts of the active substance Chlorantraniliprole in corn were below the residue limits determined in Türkiye and Europe (Chlorantraniliprole: 0.02 mg/kg), so it was concluded that the samples taken from the 14th day were reliable. Similar studies are also available. Barmota et al. (2021) reported that the residues in corn leaves were below the quantification limit (LOQ) of 0.03 mg/kg 30 days after spraying at the recommended dose.

CONCLUSION

This study aimed to evaluate the effectiveness of four doses (2, 3, 4, and 5 ml) of Chlorantraniliprole 600 g/l SC against *S. frugiperda.* It also aimed to determine the residue amount of the active substance at a dose of 5 ml/da. As a result of the analyses, it was determined that the dose of 5 ml/da of Chlorantraniliprole 600 g/l SC produced the greatest effect. In addition, no residue risk was found in the samplings. It can thus be said that a dose of 5 ml/da of Chlorantraniliprole 600 g/l SC can be used effectively against *S. frugiperda*.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

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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Putrescine and boron treatments increase seed quality in Melon (*Cucumis melo* var. *inodorus*)

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Abstract

In agriculture, seed quality and high yield are directly related to each other. Hybrid seed production is difficult and expensive, and as it were not enough, the rate of empty seeds at the end of production is high, which reveals the importance of studies in seedling emergence. Empty seed formation is frequently encountered in melons and causes low seed productivity. Efforts to eliminate this situation and improve seed germination and emergence quality will have positive effects on both producers and consumers. In this study, it was aimed to determine the effects of boron and putrescine on seed number per fruit, seed germination, and seed emergence rates in Cucumis melo var. inodorus. The seeds used in the research were produced from plants of the SR-21 and Sİ-8 genotypes to which boron and putrescine were applied in the spring-summer growing period of 2023. In research, germination and emergence rates, germination and emergence times, germination and emergence index parameters in seeds were investigated. Seed quality parameters were positively affected by boron and putrescine applications, boron increased seed quality compared to the control. Putrescine was the application that had the best effect on seed quality. As a result, it was determined that putrescine (90.16%; 92.83% respectively), boron (83.83%; 94.67% respectively), and boron+putrescine combinations (78.16%; 84.17%) increased the germination and emergence rate compared to the control group (%55, 58.17 respectively or % increases can be given compared to the control group). In conclusion, to produce higher quality seeds, breeders and seed companies could apply putrescine and boron to the plant before hand pollination.

Keywords: Boron, Putrescine, Melon, Seed

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INTRODUCTION

Melon is an annual vegetable belonging to the *Cucurbitaceae* family (Pitrat, 2016; Wan Shafiin et al., 2021) and spread around the world from Africa. Although there is no clear information about its origin and area, it can be cultivated in subtropical and tropical regions (Abraham-Juarez et al., 2018; Kesh and Kaushik, 2021). In Türkiye, 1.6 million tonnes of melons are produced in an area of 62.7 thousand hecares. The most melon is produced in the provinces of Denizli (126 000 tons), Konya (117 000 tons), Adana (116 000 tons), and Antalya (115 thousand tons) (TUIK, 2023). However, considering the effects of global climate change on Türkiye and in the world, it is important to implement practices that will both protect plants and seeds from this change and increase production and quality.

Boron, a microelement, is effective in both generative and vegetative tissues in plants and also plays an important role in cell wall formation, plant physiology, and increasing seed yield by supporting fertilization (Kumar et al., 2012). If boron is low in the soil, it causes losses in plant production areas (Shorrocks, 1997) and also negatively affects pollen formation, seed quality, and germination (Sillanpaa, 1982; Dell et al., 2002). In a study, it was stated that the low germination rate in seeds was due to the insufficient amount of boron in pollen (Pinho et al., 2010). Boron was recently applied to lentils (Khattab et al., 2016), flax (Jankowski et al., 2016), canola (Khan et al., 2016), olives (Gündeşli and Nikpeyma, 2016), peas (Sharma and Sharma, 2016), corn (Andric

et al., 2016). It has been found to increase seed yield and quality in sunflower (Silva et al., 2016), beans (Parry et al., 2016), and radish (Dev, 2010; Namlı et al., 2022). In addition, since it is known that boron increases the quality of pollen in watermelons (Adıgüzel et al., 2023d), it is thought that it may have a positive effect on the seeds what is the seed.

Putrescine is a polyamine which positively affects growth and development in plants and increases tolerance to stress conditions (Liu et al., 2015; Thomas et al., 2020; González-Hernández et al., 2022). Studies have shown that putrescine application in vegetables increases the marketing period in melon (Lester, 2000), yield and quality in onion and artichoke (Amin et al., 2011; El-Abagy et al., 2010), and prevents cold damage in cucumber (Zhang et al., 2009). In addition, in a limited number of studies on seeds, it has been reported that putrescine increases the germination and viability rates in corn (Hussain et al., 2013) and the germination rate of semi-viable seeds in melon (Adıgüzel et al., 2023c). As a result of the putrescine application in lemon, by increasing ovule longevity and ovule quality the effective pollination period was increased and at the end, seed quality and germination level were also increased (Karabıyık, 2024). When these positive effects of boron and putrescine on plants are evaluated, it is thought that seed quality would be increased if boron and putrescine are applied together. In this context, although there is a study conducted on lemon (Karabıyık, 2024) about the effects of putrescine on the seed, as our knowledge there is no study on *Cucurbitaceae* species. This study aimed to determine the seed yield, full seed rate, germination and emergence rates of seeds harvested from the fruits of plants treated with boron, putrescine, and boron+putrescine.

MATERIALS AND METHODS

This study was carried out in a greenhouse at Research Application Areas of Çukurova University Faculty of Agriculture Department of Horticulture in 2024 and the seed tests were carried out in the Seed Technologies Laboratory of the same department.

Materials

In this study, SR-21 and Sİ-8 genotypes from inodorus group melon genotypes (*Cucumis melo* var. *inodorus*) were used. The seeds used in the research were obtained as a result of self-pollination of putrescine and boron-treated SR-21 and Sİ-8 genotypes in the spring-summer growing season of 2023. The used plants were in optimum care conditions. And only one fruit was allowed for every plant.The boron and putrescine treatments were made as follows: (i) Boron application [150 g/100 L EtiDot 67 (21% boron) applied twice with the help of a foliar back sprayer when the plants are at the 6-leaf stage and the small fruit stage]; (ii) Putrescine application [at the first male flower signs begin to appear on the plants, a single application of 0.25 mM 1.4 diaminobutane dihydrochloride by spraying the plants with a back sprayer]; (iii) Boron + Putrescine [application of boron and putrescine together as stated above] (iv) Control [only water treatment].

Seed Analysis

Seeds were harvetsed from each mature fruit obtained by inbreeding and left to ferment for 3 days. After the fermentation process, the seeds were washed and left to dry. Total and full seed were counted, and seed number and fully developed seed ratio were recorded. The obtained seeds were planted separately for each genotype and treatment for germination and emergence tests in February 2024. Randomly selected full seeds obtained from 5 fruits derived from each plant were used for both germination and emergence tests. Seed germination and emergence tests were carried out according to ISTA rules (ISTA, 2007) in 4 replicates with 100 seeds in each replication. For seed germination tests, 90 mm plastic petri dishes were used, and the seeds were germinated in the petri dishes between filter paper in an oven (Memmert) at 25°C for 7 days. For seed emergence tests, river sand was used, and emergence tests were performed in the growth chamber under 16 hours of light and 8 hours of darkness conditions for 10 days. Rate, duration, and index were calculated by counting the germinating and emerging seeds every day (Ellis and Robert, 1980; Demir et al., 2008).

Statistical Analysis

The experiment was set up according to the randomized design with 3 replications and statistical analyses were carried out according to randomized plots factorial experimental design on the basis of varieties and applications, each period within itself. The data were subjected to variance analysis in the JMP 13.2.0 package program and the differences between the means were classified at the 5% significance level according to the LSD test. Arc-sin transformation was applied to percentage values.

RESULTS AND DISCUSSION

The number of seeds harvested from fruits is given in Table 1. The total number of seeds was singificanty affected by genotype, treatment, and genotype x treatment interaction. In general, Sİ-8 (688.5 seed/fruit) produced more seeds than the SR-21 (527.5 seed/fruit) genotype, and all treatments increased the total number of seeds by approximately 30% compared to the control. The highest increase was obtained from boron + putrescine treatment (826.3 seed/fruit) to the Sİ-8 genotype and the lowest number of seeds was obtained from the control treatment to the SR21 genotype (477.7 seed/fruit).

Although the number of seeds is important in breeding studies, the high rate of full seeds provides information about the accuracy of the pollination process. The full seed rates obtained from the combinations used in the study are given in Table 1. The highest fully developed seed rate was obtained by applying putrescine to SR-21 genotype as 92.86% and the lowest was found to be 64.82% when both boron and putrescine were applied to Sİ-8. The SR-21 genotype (83.95%) had more full seeds than Sİ-8 genotype (70.80%) and the putrescine treatment increased the rate of fully developed seeds.

Table 1. Effect of Boron and Putrescine Treatments on Total Seed Number and Full Seed Rate of Different Genotypes.

Application	Total Seed Number (seed/fruit)			Full Seed Rate (%)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	477.7 e	543.0 de	510.3 B	87.08 b	67.80 ef	77.44 B
Boron	593.7 cd	678.7 bc	636.2 A	73.93 d	78.69 c	76.31 BC
Putrescine	527.7 de	706.0 b	616.8 A	92.86 a	71.88 de	82.37 A
Boron+ Putrescine	511.0 de	826.3 a	688.7 A	81.94 c	64.82 f	73.38 C
Genotype average	527.5 B	688.5 A		83.95 A	70.80 B	
$LSD_{(Genotype)}$:43.07 ***; $LSD_{(Application)}$: 60.92*** *; $LSD_{(Genotype\ x\ Application)}$: 86.15**				$LSD_{(Genotype)}$: 1.53***; $LSD_{(Application)}$: 2.16***; $LSD_{(Genotype\ x\ Application)}$: 3.05***		

NS: Not Significant; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application. And arc/sin transformation was made to percentage values.

The data on seed germination and emergence rates were given in Table 2. The table showed that the differences between genotypes were not statistically important while it was significant in terms of treatment and genotype x treatment showing that the treatments can be effective for all melons. In this context, when the treatment averages were evaluated, the highest value was obtained from putrescine with 90.16%, followed by boron application with 83.33%. The lowest value was obtained from the control application as 55.00%.

In terms of seed emergence rates, it was determined that the differences between the data were non-significant on the basis of genotypes, but important in terms of treatment and genotype x treatment interaction as it was in germination tests. The data showed that the highest values were obtained from boron and putrescine applications with 94.67% and 92.83%, respectively. At the same time, the lowest value in the mantioned parameter was again in the control application with 58.17%.

In this study, the effects of putrescine and boron application were examined, putrescine (90.16%; 92.83%, respectively), boron (83.83%, 94.67%, respectively) and boron it was found to increase the rate of the results are consistent with other studies.

The results showed that the application of putrescine and boron to melon plants increased seed germination and emergence rates.

Table 2. Effects of Boron and Putrescine Treatments on Seed Germination and Emergence Rate in Seeds of Different Genotypes (%).

Application	Seed Germination Rate (%)			Seed Emergence Rate (%)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	70.00 c	40.00 d	55.00 C	61.66 d	54.67 d	58.17 C
Boron	85.00 ab	82.67 bc	83.83 AB	98.67 a	90.66 bc	94.67 A
Putrescine	85.00 bc	95.33 a	90.16 A	88.33 c	97.33 ab	92.83 A
Boron+ Putrescine	81.33 bc	75.00 bc	78.16 B	80.00 c	88.33 c	84.17 B
Genotype average	80.33	73.25		82.17	82.75	
$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: 8.34***; $LSD_{(Genotype\ x\ Application)}$: 12.07*				$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: 0.69***; $LSD_{(Genotype\ x\ Application)}$: 9.75**		

NS: Not Significant; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application. And arc/sin transformation was made to percentage values.

When the seed germination time was examined, it was determined that there was no significant change between the periods and seed germinated within 3-4 days in all applications (Table 3). When looking at the seed emergence times, the differences were not statistically significant, but the longest seed emergence time was in the control treatment (5.56 days) and the shortest in the putrescine (4.09 days). Considering the seed emergence times, the latest emergence was observed in the control group (7.00 days) in SR-21, while all other treatments were statistically in the same statistical group and emerged between 3.66-4.71 days.

Table 3. Effects of Boron and Putrescine Treatments on Seed Germination and Emergence Time in Seeds of Different Genotypes (day).

Application	Seed Germination Time (day)			Seed Emergence Time (day)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	3.26	3.00	3.13	7.00 a	4.12 b	5.56
Boron	3.00	3.03	3.02	4.71 b	4.79 b	4.75
Putrescine	3.00	3.00	3.00	3.66 b	4.52 b	4.09
Boron+ Putrescine	3.03	3.00	3.00	4.27 b	4.43 b	4.35
Genotype average	3.06	3.01		4.91	4.46	
$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: N.S.; $LSD_{(Genotype\ x\ Application)}$: N.S.				$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: N.S.; $LSD_{(Genotype\ x\ Application)}$: 1.75*.		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application.

The results of germination and emergence speed index are given in Table 4. When the seed germination and emergence speed index data were examined, it was determined that the differences were statistically significant in terms of genotype, application, and genotype x application interaction.

The germination speed index was higher in the Sİ-8 genotype (6.08) than in the SR-21 genotype (5.84). In terms of treatment averages, the highest value was recorded in the putrescine application with 7.85, and the lowest value was obtained from the control application with 2.77, meaning that the germination speed index was increased by approximately 35% with this application. In genotype x application interaction, the values range between 1.01 (SR-21 x control) and 8.22 (SR-21 x boron) in the SR-21 genotype, and between 4.53 (Si-8 x control) and 8.20 (Si-8 x putrescine) in the Si-8 genotype.

When the emergence speed index was examined, it was determined that the highest values were in the SR-21 genotype (4.93) and the lowest values were in the Sİ-8 (4.09). The treatment averages showed that the highest values were in the boron (6.12) and putrescine (6.23) treatments and the lowest seed speed index values were again in the control group (1.33). In application and genotype combinations, SR-21 x boron (7.73) and SR-21 x putrescine (7.26) had the highest values, and SI-8 x control (1.96) and SR-21 x control (0.30) had the lowest values (Table 4).

Table 4. Effect of Boron and Putrscine Treatments on Seed Germination and Emergence Speed Index of Different Genotypes.

Application	Germination Speed Index			Emergence Speed Index		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	1.01 d	4.53 c	2.77 C	0.30 d	1.96 c	1.33 C
Boron	8.22 a	7.00 b	7.61 AB	7.73 a	4.50 b	6.12 A
Putrescine	7.50 ab	8.20 a	7.85 A	7.06 a	7.40 b	6.23 A
Boron+ Putrescine	6.63 b	7.50 ab	7.06 B	4.63 b	4.50 b	4.57 B
Genotype average	5.84 B	6.08 A		4.93 A	4.09 B	
$LSD_{(Genotype)}$: 0.46***; $LSD_{(Application)}$: 0.65***; $LSD_{(Genotype\ x\ Application)}$: 0.92***				$LSD_{(Genotype)}$: 0.50**; $LSD_{(Application)}$: 0.70***; $LSD_{(Genotype\ x\ Application)}$: 0.99***		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application.

Studies have shown that boron increases the pollen germination rate and the velocity of pollen germination (Goldberg et al., 2003; Şensoy et al., 2003; Ansari and Chowdhary, 2018; Fang et al., 2019; Hidayat et al., 2019, Adıgüzel et al., 2022; Adıgüzel et al., 2023d) which in turn provides a more efficient fertilization process. In

studies conducted on lentils (Khattab et al., 2016), flax (Jankowski et al., 2016), canola (Khan et al., 2016), olives (Gündeşli and Nikpeyma, 2016), and peas (Sharma and Sharma, 2016) boron treatment increased the seed germination rate. It has also been stated that there was a significant rise in the number of seeds in corn (Andric et al., 2016), sunflower (Silva et al., 2016), beans (Parry et al., 2016) and radish (Dev, 2010; Namlı et al., 2022) by boron applications.

In the presence of putrescine, the ovule longevity was increased (Akbaş ve Solmaz, 2019; Erol ve Sarı, 2019; Solmaz and Yıldız, 2020; Karabıyık, 2024), providing the pollen tube to reach the ovule in a longer period. At the same time, putrescine provides plants to overcome some stress conditions like salt (Ekinci et al., 2019; Yuan et al., 2019; Islam et al., 2020), temperature (Zhang vd., 2009; Palma et al., 2016; Lu et al., 2022; Sharma et al., 2023) and drought stresses (Farsaraei et al., 2021; Li et al., 2021; Ma et al., 2022). The effects of putrescine on plants, fruit, and seeds were reported in apples (Costa et al., 1985), lemons (Karabıyık, 2024), onions (Amin et al., 2011), etc. before. In the light of this knowledge for boron and putrescine, it could be said that by increasing fertilization efficiency and pollen-ovule interaction, there could be stronger seeds with higher germination capacity. In recent studies, the application of 20 and 50 ppm putrescine on pepper plants increased germination and root capacity (Khan et al., 2016). Koç et al. (2014) reported that in the presence of putrescine, the germination rate was increased in bean genotypes exposed to salt stress by repressing the negative effects of salt stress. Moreover, in a previous study in melon seeds, the germination was increased by approximately 40% with putrescine dipping treatment even in semi-viable seeds (Adıgüzel vd., 2023c). In another study conducted on lemon, it was determined that 0.05 and 0.1 m Mol putrescine treatment to plants increased the seed germination rate by approximately 40% (Karabıyık, 2024). In this study conducted on melon genotypes, seed germination was increased by approximately 63% with the application of putrescine to melon plants. At the same time, this increase was 50% in boron and 42% in boron+putrescine treatment compared to the control group. So, it could be easily said that putrescine only or boron and putrescine in a couple could have a constant effect on the germination and emergence performance of melons. Seed number and quality affects from environment (Alqudah et al., 2011; Pervez et al., 2009; Sehgal et al., 2018), genotype (Jat et al., 2016), grafting (Suárez-Hernández et al., 2022; Adıgüzel et al., 2023b; Alam et al., 2023), flower sex (Adıgüzel et al., 2023b) and different treatments (Kuzucu and Dumlupınar, 2017; Ozdemir et al., 2019; Acharya et al., 2020; Alam et al., 2022; Yousef, et al., 2023). In another study of our team, it was reported that the total number of seeds varied between 773 and 291 number the rate of fully developed seeds varied between 36% and 97% which was thought as this higher numbers was due to the rapid advancement of pollen tubes to the ovules, depending on the flower sex structure (Adıgüzel et al. 2023b). In addition, it has been concluded that healthy seed formation is not caused by pollen, but is related to the homogeneous development of seed ovules. In this study, the total number of seeds increased compared to the control, and putrescine treatment came to the fore in the proportion of fully developed seeds. Putrescine has effects on ovule longevity (Karabıyık, 2024), reducing stress factors (Thomas et al., 2020), ensuring the development of healthy male and female flowers (Singh et al., 2014) and accordingly increasing fruit quality (Sayyad-Amin et al., 2018). As a result of the study, the highest seed and fully developed seed ratio was obtained from putrescine treatment. This proves that putrescine can produce healthy seeds.

Seed germination and emergence time analysis is an important parameter as it provides information about how many days it takes for the seeds to germinate (Ranal and Santana, 2006; Sarma, 2024). In this way, information is obtained about whether the seeds germinate faster or slower depending on their genetic structure, age, or treatments. In this case, ensuring earliness, especially in obtaining seedlings, is proportional to the germination time of the seeds. The emergence time of a seed depends on the availability of reserves within the seed (Taiz and Zaiger, 2002). The emergence time was between 7 and 10 days; the germination time was between 2 and 7 days in recent studies (Adıgüzel et al., 2023a, Adıgüzel et al., 2023b). The parameter in question in this study varied between germination time 3.00 and 3.26; emergence time 3.66 and 7.00 days. In a study conducted on viable and semi-viable seeds, the dipping of melon seeds to putrescine solution did not provide any significant increase (Adıgüzel et al., 2023c). However, in this study, there was a difference of approximately 1.5 days due to the emergence time of root tip in the emergence tests. So, it could be concluded that the increase of emergence time could be originated from the inner putrescine level of the plant with the efficient pollination and fertilization process of the flower. It was also concluded as the reserves could be accumulated in the seeds with putrescine applications and strengthen the seed. However, this opinion needs more detailed study.

The germination and emergence speed index shows the initial strength of the seed (Copeland and McDonald, 2012). In addition, seeds lose their strength vigor at the stage before losing their ability to germinate (Sivritepe, 2012). In this sense, germination and emergence speed index are important in determining seed quality. In this study, compared to control, the germination speed index increased 3 times with putrescine, boron, and boron x putrescine treatments and the emergence rate increased approximately 4-6 times. From this, it can be concluded that the germination index of putrescine acting on the mother plant will increase significantly, thus stronger seeds that provide emergence in a shorter time can be obtained. In general, the seeds that were initially strongest in terms

of germination and viability were obtained by applying putrescine and boron treatments which had higher values than the control. In a study conducted on apple (Naija et al., 2009) and olive (Rugini and Mencuccini, 1985) it was showed that the increase in inner putrescine content increases the root formation in the plant and in the scions indicating that the putrescine has an effect on root initiation process.

In this study, the seed germination capacity in terms of germination and emergence rates, germination and emergence times, and germination and emergence speed index were increased by putrescine and/or boron treatments. The results of this study and recent studies together showed that this result originated from better plant fertilization success with superior and rapid root formation and in turn this shows a better germination capacity with putrescine and boron treatments.

CONCLUSION

In this study, where the effects of boron and putrescine applications on melon plants were examined, it has been determined that the seed germination capacity is affected by putrescine and boron treatments. In this context, although boron had a greater effect on seed germination, it still had an increase in germination rate than control treatments. Putrescine was the most effective treatment that increased the total number of seeds and the rate of filled seeds. Considering that the treatments strengthen the ovules and support the development of the pollen tube, it is thought that the embryos formed inside the seeds should also be quite developed. Thus, germination and emergence rates in this study were significantly higher than the control treatment, which supports this idea. In conclusion, putrescine treatment for the plant is an effective treatment for seedling growth by increasing the seed quality.

For a more profitable production, growers and seedling producers can be advised to spray their plants with 0.25 m Mol putresin and the specified amount of boron.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there are no actual, potential or perceived conflicts of interest for this research article.

Author contribution

P. A and Ş. K: Study data analysis, conceptualization and design of the research; P. A: Wrote the original draft; and İ. S: Editing and preparation of the manuscript. All authors have read and approved the manuscript.

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Improvement of reproductive and growth characteristics of local hair goats raised in extensive system in Kahramanmaraş province

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Abstract

This study aims to ensure the profitability of the enterprise and the sustainability of production by increasing the number of populations with high breeding value in Hair goat farms producing under extensive conditions. For this purpose, the reproductive criteria for the goats were determined and data on growth performance and survival rate of the kids were recorded on an annual basis. The current study was started in 2018 with 5175 female goats and 257 male goats with the voluntary participation of 26 breeders of hair goats in Dulkadiroğlu district of Kahramanmaraş province and was conducted over five years. The morphological characteristics of the hair goat breed and the birth and weaning weights of the kids were taken into account when selecting the breeding stock to be included in the herd each year. Approximately 10% of the male kids and 50% of the female kids with the best growth and breeding characteristics were selected for breeding. At the end of the study, the birth rate was found to be between 49.77 and 63.75%, the twinning rate was between 18.33 and 25.98%, prolificacy was between 58.90 and 80.08% and the litter size was between 1.18 and 1.26. The birth weight of the kids was found to be between 2.97 kg and 3.11 kg, the weaning weight were between 11.30 kg and 14.96 kg, the daily body weight gain to weaning age were between 92.54 and 131.64 g and the survival rate was between 91.89% and 95.68%. Based on these results, it was found that the differences in the growth traits of the kids according to year were significant ($P<0.001$). It was also found that the growth performance of male and single-born kids were significantly higher than those of female and twin-born kids depending on sex and birth type ($P<0.001$). As a result, it was found that keeping records based on some performance values in production with local genetic resources grown in an extensive system and keeping offspring with high breeding value in the herd based on these records helps to increase production performance due to the improvement of genetic structure.

Keywords: Hair goat, Breeding, Reproductive criteria, Growth performance, Survival rate

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INTRODUCTION

Goats, bred in a very wide world geography, have been an important source of food and consumption throughout human history. As land and climatic conditions become difficult, goats are becoming one of the most important sources of income for the poor, and their breeding is practiced in traditional production methods and under extensive conditions (Gökdal et al., 2013). When considering the goat population in Türkiye over the last 20 years, it can be seen that the goat population, which was around 7 million animals in 2001, showed a declining trend between 2001 and 2010, but increased again between 2010 and 2024, reaching around 10.5 million animals today (TÜİK, 2024). In Türkiye, animal production is not sufficient to meet the food needs of the constantly growing human population. This situation is also reflected in the recent rise in meat prices. It is necessary to maximize the use of all types of livestock that are a source of human nutrition. Among these resources, Hair goat

breeding has an important place in livestock production with a share of about 96% of the Turkish goat population, which is active in almost all regions of Türkiye. The most important factors for the spread of Hair goats throughout Türkiye include adaptability to all kinds of climatic and terrain conditions in Anatolia, ability to be raised under inadequate care and feeding conditions, strong physique, resistance to diseases and resistance to heat and cold (Koluman et al., 2024). It is also a very valuable breed when it comes to converting plant products and/or waste into valuable animal foods. It is grown almost inexpensively on heaths, shrubs, forest pastures, stubble and fallow grasses in villages and hamlets in and near forests at high altitudes. However, since Hair goat breeding in Türkiye is mainly practiced in rural areas and under an extensive production model, the yield of the goats remains low (Koluman et al., 2024). The value of Hair goats in the national dimension is important and there is a need for studies to improve their yield characteristics. Therefore, the number of studies on increasing the meat and milk yield of herds reared under extensive conditions in Turkey has increased in recent years. In this context, the present breeding study conducted in the province of Kahramanmaraş for hair goats, which are reared under extensive conditions in rural areas and represent an important genetic resource for Turkey, is important. The Mediterranean region, where Kahramanmaraş province is located, is the most important goat breeding region of Türkiye due to its mountainous landscape and climatic characteristics, and the proportion of Hair goats in the goat population is about 27-28% (Ertuğrul et al., 2000; Koluman et al., 2024).

The aim of this study was to increase the yield by creating an elite herd with high breeding value through selection from the existing Hair goats raised by the farmer and to increase the profitability of the enterprise accordingly.

MATERIALS AND METHODS

Publication authorization for this study was obtained from the Directorate-General for Agricultural Research and Policy byletter dated 24.05.2024 and numbered 14390928. The current study on Hair goats was started in 2018 in the province of Kahramanmaraş in the Eastern Mediterranean with 5175 animals of female goats and 257 male animals of breeding goats and continued for five years. For this purpose, 26 Hair goat breeders registered in the database of Sheep and Goat Breeders Association and breeding in extensive production system were voluntarily selected to represent Dulkadiroğlu district of Kahramanmaraş province. Accordingly, the present study was conducted in 10 neighborhoods of Dulkadiroğlu district (latitude: 37.5833; longitude: 36.9333) of Kahramanmaraş province (Figure 1). The region where the study was conducted reflects the climatic conditions of the Mediterranean region with hot and dry climate conditions in summer and mild and rainy climate conditions in winter.

Figure 1. Map image of Kahramanmaraş Dulkadiroğlu district

Table 1 shows the number of neighborhoods and animals identified for the study in the Dulkadiroğlu district. In the production herds on the farms, the mating process was conducted by free mating, resulting in a ratio of one male for every 25 females. The female members of the herd remained in the population until they were no longer

fertile, while the males were replaced every two years when the first offspring of their sons reached sexual maturity. Surplus male offspring and bucks that had spent two years in the herd were sold that year.

Table 1. Number of neighborhoods and animals identified for the study in the Dulkadiroğlu district

District	Neighborhoods	Number of breeder	Number of does	Number of bucks
Dulkadiroğlu	Budaklı	10	2190	109
Dulkadiroğlu	Başdervişli	2	260	13
Dulkadiroğlu	Hacıeyüpoğlu	1	330	17
Dulkadiroğlu	Bahçeli	2	475	23
Dulkadiroğlu	Eyüpsultan	2	380	19
Dulkadiroğlu	Bulanık	2	400	20
Dulkadiroğlu	Kuzucak	4	650	32
Dulkadiroğlu	Dereköy	1	140	7
Dulkadiroğlu	Dereli	1	130	6
Dulkadiroğlu	Elmalar	1	220	11
Total		26	5175	257

While the male breeding animals were exchanged among the farmers, the remaining male kids were fattened and sold. The goats were mated according to the breeding plan determined by the breeders. However, the bucks were only brought together with the does during the planned mating season, which lasted from August to September. In this way, the reproductive capacity of the bucks could be optimally utilized. After recording some reproductive criteria after birth in all herds between 2018-2022 on the farms, performance data such as birth weight, mortality rate and weaning weight were measured. After the colostrum consumption of the born kids was ensured, the birth weight was recorded by the breeders using a digital scale. While the birth records were taken, the sex, birth type and birth dates of the kids were also recorded. The weaning weights of the kids were made when they were 135 days old on average. The 135th day live weights of all kids were calculated individually using the interpolation method, based on the daily body weight gain they gained between birth and the second weighing. The daily body weight gain was found by dividing the total weight gain between the two weighings by the age on the weighing day. The survival rate of the kids was calculated by dividing the number of kids who survived to weaning age by the number of kids born alive. Breeding animals were selected according to the needs of the farm, taking into account descriptive traits such as morphological traits and some performance data of the kids. In accordance with the above criteria, approximately 10 percent of the males and 50 percent of the females with the most optimal growth and reproductive traits were selected as core herds. In addition, to investigate the reproductive criteria, the following formulas were used to calculate kidding rate (%), twinning rate (%), litter size and prolificacy (%).

Birth rate = (Number of does kidding/Number of mated does) x100

Twinning rate = (Number of twin births/Number of does kidding) x 100

Litter size = Number of born kids/Number of does kidding

Prolificacy = (Number of born kids/Number of mated does) x 100

On all farms, the animals were fed around 500-600 g of concentrated feed and around 1000-1100 g of low-quality roughage per animal per day for three months during the winter months. In the other seasons, the animals were fed on pasture for nine months. The study data were analyzed using the SPSS package program (SPSS, 2021) and the mean values of two groups were compared using the t-test. A one-way ANOVA test was used to compare the means of more than two groups. The mathematical model of the one-way ANOVA is as follows. A comparison of the differences between the groups was conducted using the Duncan test. Data were summarized as mean and standard error. All tests were performed with a statistical significance level of 0.05.

$Y_{ij} = \mu + \alpha_i + e_{ij}\ i = 1, 2, \ldots, t\ j = 1,2, \ldots, r$

Where μ is the mean effect, αi is the i^{th} year effect, t is number of treatment, r is the number of replications, eij is the error term (Montgomery, 2001).

RESULTS AND DISCUSSION

Reproductive Characteristics in the Hair Goats

The results on the reproductive traits of Hair goats are presented in Table 2 according to the years in which the data were collected. The results on reproductive traits showed a birth rate of 49.77 to 63.75%, a twinning rate of 18.33 to 25.98%, prolificacy of 58.90 to 80.08% and a litter size of 1.18 to 1.26%. When examining the reproductive perfomance of goats, an improvement was recorded in all subsequent years compared to the first year of the study.

Table 2. Reproductive characteristics of Hair goats by year

Reproductive characteristics	Years				
	2018	2019	2020	2021	2022
Number of mated does	4800	4805	4795	5175	5175
Number of given birth does	2389	3011	3048	3201	3299
Number of twin births	438	610	792	752	796
Number of born kids	2827	3621	3840	3953	4095
Birth rate (%)	49.77	62.66	63.57	61.86	63.75
Twinning rate (%)	18.33	20.26	25.98	23.49	24.13
Prolificacy (%)	58.90	75.36	80.08	76.39	79.13
Litter size	1.18	1.20	1.26	1.23	1.24

In some studies on Hair goats, the birth rate was reported as 79.00% by Şengonca et al. (2003), 82.93% by Tozlu (2006), 90.00% by Şimşek et al. (2006), 80.00% by Erişir and Gürdoğan (2004) and 85.89% by Erten and Yılmaz (2013). On the other hand, Prolificacy was reported as 100.00% by Çam et al. (2003), 79.00% by Şengonca et al. (2003), 116.00% by Erişir and Gürdoğan (2004), 103.00% by Tozlu (2006), 118.00% by Şimşek et al. (2006), 96.27% by Oral and Altınel (2006) and 101.28% by Erten and Yılmaz (2013). In the present study, the birth rate and prolificacy were found to be low compared to the literature. This result shows that goats on the farms not become pregnant at a significant rate as a result of mating. It is thought that this situation is due to the fact that farms operating in the extensive system in rural areas do not apply the selection and elimination practices that should be carried out each year in about 20-22%. In the following years of the study, however, by eliminating animals that had lost their reproductive and selecting offspring from dam goat with high breeding value for the herd, improvement in the birth rate and prolificacy was achieved. The twin birth rate was reported as 32.56% by Erişir and Gürdoğan (2004), 17.65% by Tozlu (2006) and 17.91% by Erten and Yılmaz (2013). The results of the current study in terms of twin birth rate are largely consistent with the literature, although an increase was observed in all other years compared to the beginning of the study. Litter size was reported as 1.43 by Özcan (1977), 1.17 by Tozlu (2006), 1.41 by Şimşek et al. (2006) and 1.18 by Erten and Yılmaz (2013). In the current study, the litter size was found to be within the literature limits, lower than the results of Özcan (1977) and Şimşek et al. (2006) but higher than the results of the studies conducted by Tozlu (2006) and Erten and Yılmaz (2013). Although it is assumed that the reproductive performance of these goats reared in the extensive system is lower than that in the intensive farms, it has been shown that the reproductive performance of the Hair goats reared in farmer condition will increase with the effect of the breeding program carried out.

Growth characteristics and survival rate of hair goat kids by year

Birth weight, weaning weight, daily body weight gain to weaning and survival rates were recorded for five years in relation to growth characteristics of female and male kids (Table 3). Accordingly, the birth weights of the kids ranged from 2.97 kg to 3.11 kg, weaning weights ranged from 11.30 kg to 14.96 kg, daily body weight gain ranged from 92.54 to 131.64 g and survival rate to weaning ranged from 91.89 to 95.68%. The observed differences in the growth-related performance values of the Hair goat kids were significant depending on the year (P<0.001).

Table 3. Growth characteristics of Hair goat kids

Years	Birth weight (kg)	Weaning weight (kg)	Daily body weight gain (g)	Survival rate (%)
2018	2.97±0.01^{a}	11.30±0.09^{a}	92.54±0.96^{a}	91.89
2019	2.99±0.01^{a}	12.14±0.08^{b}	101.68±0.86^{b}	95.36
2020	3.03±0.01^{b}	14.86±0.07^{d}	131.50±0.83^{d}	95.68
2021	3.10±0.01^{c}	13.11±0.8^{c}	111.18±0.86^{c}	94.46
2022	3.11±0.01^{c}	14.96±0.5^{d}	131.64±0.51^{d}	94.11
P value	P<0.001	P<0.001	P<0.001	-

In some studies, the birth weight of Hair goat kids was reported as 3.89 kg by Darcan (2000), 2.60 kg by Öztürk (2000), 3.80 kg by Daş and Savaş (2002), 2.70 kg by Çam et al. (2003), 2.63 kg by Şengonca et al (2003), 2.99 kg by Şimşek (2005), 3.31 kg by Karadağ (2006), 3.72 kg by Tozlu (2006), 2.58 kg by Oral and Altınel (2006), 2.18 kg by Şimşek et al. (2006), 3.12 kg by Atay et al. (2010), 3.01 kg by Erten and Yılmaz (2013), 2.75 kg by Gökdal et al. (2013), 3.70 kg by Çelik and Olfaz (2018) and 3.11 kg by Alşahan and Öztürk (2019). The results of this study are within the scope of the literature results and are similar to the results of the studies of Alşahan and Öztürk (2019) and Atay et al (2010). In addition, it was found to be lower than the results of the studies of Darcan (2000), Daş and Savaş (2002), Karadağ (2006), Çelik and Olfaz (2018) and Tozlu (2006) and higher than the results of other studies. The weaning weights of three-month-old kids were reported as 11.84 kg by Cengiz et al (1995), 18.00 kg by Darcan (2000), 13.70 kg by Çam et al (2003), 17.77 kg by Şimşek (2005), 13.58 kg by Oral and Altınel (2006), 16.0 kg by Tozlu (2006), 16.05 kg by Şimşek and Bayraktar (2007), 17.02 kg by Erduran and

Yaman (2012) and 12.32 kg by Erten and Yılmaz (2013). In this study, weaning weights of kids were lower than the results of the studies by Darcan (2000), Şimşek (2005), Tozlu (2006), Şimşek and Bayraktar (2007) and Erduran and Yaman (2012), but similar to the results of other studies. The survival rate of Hair goat kids to weaning reported 93.34% by Eser (1998), 78.16% by Odabaşıoğlu and Altın (1992), 78.61% by Şengonca et al (2003), 93.3% by Çam et al (2003), 82% by Şimşek (2005), 88.11% by Tozlu (2006), 95.44% by Oral and Altınel (2006), 90.62% by Şimşek and Bayraktar (2007), 80.00% by Erduran and Yaman (2012), 89.87% by Erten and Yılmaz (2013), 89.27% by Tekin and Arlı (2019), 94.91% by Elmaz et al. (2020), 95.7% by Ceyhan et al. (2022) and 93.48% by Alkkonyak and Güngör (2022). In the present study, the survival rate was higher than in Odabaşıoğlu and Altın (1992), Şengonca et al. (2003), Şimşek (2005), Tozlu (2006), Erduran and Yaman (2012), Erten and Yılmaz (2013), Tekin and Arlı (2019), but it was similar to the results of other researchers. The growth performance and survival rates of the kids generally improved in the later years compared to the year in which the study began. This situation can be explained by the fact that the offspring of breeding animals with high reproductive are kept in the herd by the breeders. However, it should be noted that this method does not directly reflect the relationship between the genetic predisposition of the parents to growth and performance traits and the likelihood of their occurrence in the offspring.

Growth characteristics and survival rate of hair goat kids according to dam age, sex and birth type

The growth characteristics of Hair goat kids depending on dam age, the sex and type of birth of the kids are shown in Table 4 as an average of all years. Depending on the dam age, the birth weights of the kids varied between 2.94 kg and 3.07 kg, the weaning weight between 12.23 kg and 14.67 kg, the daily body weight gain until weaning between 102.44 and 130.30 g and the survival rate between 88.38% and 96.93%. The birth weight, weaning weight and daily body weight gain to weaning depending on sex were 3.12 kg; 13.98 kg and 120.71 g in male kids and 2.94 kg; 12.42 kg and 105.30 g in female kids, respectively. The birth weight, weaning weight, daily body weight gain to weaning depending on birth type were 3.14 kg; 13.35 kg and 113.51in single kids and 2.66 kg; 12.77 kg and 112.52 g in twinning kids, respectively.

Table 4. Growth characteristics and survival rate of Hair goat kids depending on dam age, sex and birth type

Dam age	Birth weight (kg)	Weaning weight (kg)	Daily body weight gain (g)	Survival rate (%)
1	2.94±0.03^{a}	14.67±0.34^{a}	130.30±3.81^{a}	88.38
2	3.01±0.01^{b}	13.01±0.07^{cd}	111.09±0.76^{cd}	93.38
3	3.06±0.01^{b}	13.62±0.07^{b}	117.39±0.81^{b}	94.26
4	3.07±0.01^{b}	13.25±0.07^{c}	113.17±0.73^{c}	94.12
5	3.02±0.01^{b}	13.28±0.09^{c}	113.92±0.99^{c}	95.02
6	3.03±0.02^{b}	12.86±0.11^{d}	109.25±1.24^{d}	95.15
7≤	3.01±0.02^{b}	12.23±0.10^{e}	102.44±1.09^{e}	96.93
P value	P<0.001	P<0.001	P<0.001	-
Sex				
Male	3.12±0.01	13.98±0.04	120.71±0.49	94.65
Female	2.94±0.01	12.42±0.05	105.30±0.57	94.21
P value	P<0.001	P<0.001	P<0.001	-
Birth type				
Single	3.14±0.01	13.35±0.04	113.51±0.44	94.67
Twin	2.66±0.01	12.77±0.07	112.32±0.76	93.69
P value	P<0.001	P<0.001	0.176	-

The effect of dam age on growth performance of kids was significant (P<0.001). In addition, the growth-related performance data of the kids were found to be significantly influenced by birth type and sex (P<0.001). In a study conducted by Erten and Yilmaz (2013), the birth weight of kids born from dam 2, 3 and 4 years and older was 2.81, 3.12 and 3.10 kg; weaning weight (90-day live weight) was 11.65, 12.68 and 12.65 kg; daily body weight gain to weaning was 95.95, 105.71 and 105.88 g; survival rate was 82.75%, 100.00% and 88.00%, respectively. In the same study, the birth weights of the kids depending on single and twin births were 2.97 and 3.05 kg; weaning weights were 12.52 and 12.13 kg; daily body weight gain to weaning was 105.76 and 99.27 g; survival rates were 89.09 and 91.66%, respectively. The birth weights of the male and female kids were 2.99 and 3.03 kg; the weaning

weights were 12.41 and 12.23 kg; the daily body weight gain until weaning was 104.57 and 100.46 g; the survival rate was 90.90 and 88.57, respectively. In a similar study by Ceyhan et al. (2020), the birth weight of kids born from 2, 3 and 4 years and older dam was 2.57, 2.47 and 2.53 kg; the weaning weight (90-day live weight) was 13.30, 13.57 and 13.87 kg, respectively. In the same study, the birth weights of kids depending on single and twin births were 2.71 and 2.33 kg; weaning weights were 13.76 and 13.60 kg, respectively. The researchers reported that the birth weights of male and female kids were 2.56 and 2.48 kg; weaning weights were 13.89 and 13.46 kg, respectively. Tozlu (2006) reported that the birth weights of kids born from 3, 4, 5, 6 and 7 years and older dam was 4.03, 3.68, 4.05, 3.68 and 3.40 kg; weaning weight (75-day live weight) was 15.88, 16.57, 17.11, 16.23 and 15.21 kg; daily body weight gain to weaning was 158.29, 169.40, 176.00, 165.20 and 157.88 g, respectively. In the same study, the birth weights of the kids in single and twin births was reported as 3.90 and 3.31 kg; weaning weights were 16.99 and 13.91 kg; daily body weight gain to weaning was 173.58 and 141.56 g, respectively. In addition, the birth weights of male and female kids was reported as 3.76 and 3.67 kg; weaning weights were 16.44 and 15.22 kg; daily body weight gain to weaning was 168.72 and 153.50 g, respectively. In a similar study by Tekin and Arlı (2019), the birth weight of kids born from 2, 3, 4, 5, 6 and 7 years and older dam was 3.30, 3.41, 3.51, 3.52, 3.55 and 3.57 kg; the weaning weight (120-day live weight) was 22.53, 22.51, 22.75, 22.63, 23.18 and 22.29 kg; the daily body weight gain to weaning was 158.20, 158.00, 159.90, 158.90, 163.50 and 156.10 g, respectively. The birth weight of the kids depending on single and twin births were stated 3.60 and 3.35 kg; the weaning weight was 22.86 and 22.43 kg; the daily body weight gain until weaning was 160.90 and 157.30 g, respectively. Researchers reported that birth weights of male and female kids to be 3.40 and 3.55 kg; weaning weights to be 23.57 and 21.72 kg; daily body weight gain to weaning to be 166.80 and 151.40, respectively. In another study by Elmaz et al. (2020), the birth weights of kids born from 2, 3, 4, 5 and 6 years and older dam was 3.14, 3.24, 3.56, 3.43 and 3.45 kg; the weaning weights (90-day live weight) was 17.29, 17.31, 17.58, 17.72 and 17.82 kg, respectively. In the same study, the birth weight of the kids was 3.44 and 3.21 kg depending on single and twin births; the weaning weight was 17.63 and 17.46 kg, respectively. In addition, the birth weights of the male and female kids were 3.52 and 3.13 kg; the weaning weights were 18.50 and 16.59 kg, respectively. In a similar study by Alkoyak and Güngör (2022), the birth weights of kids born from 2, 3, 4, 5, 6 and 7 years and older dam was 2.81, 2.75, 2.80, 2.81, 2.78 and 2.88 kg; weaning weight (90-day live weight) was 15.39, 15.32, 15.25, 15.77, 15.87 and 15.96 kg; daily body weight gain to weaning was 139.46, 138.70, 137.88, 143.73, 144.79 and 145.77g, respectively. The birth weights of kids in the same study was 2.85 and 2.76 kg, according to single and twin births; weaning weights was 15.73 and 15.45 kg; daily body weight gain to weaning was 143.28 and 140.17 g, respectively. The researchers reported that the birth weights of the male and female kids were 2.81 and 2.79 kg; weaning weights were 16.00 and 15.19 kg; the daily body weight gain to weaning was 46.22 and 137.22 g, respectively. In the present study, it was found that the performance values of kids depending on the dam age, birth type and sex were similar to the results reported by Erten and Yılmaz (2013) and Ceyhan et al. (2020), while they were lower than the results reported by other researchers. In addition, the survival rate of kids was found to increase with increasing dam age. This may be explained by the fact that older mothers tend to be more accepting and experienced in raising their kids. At the same time, depending on the sex and type of birth, the birth weight of male and single-born kids is higher than that of females and twins, which increases their survival rate and led to higher performance values.

CONCLUSION

Hair goats will be an indispensable gene source for the sustainability of livestock production if freshwater and feed resources are not sufficient in the near future due to climate change. Hair goats are of great value due to their status as an local gene source, which is common in almost all regions of Türkiye and is mainly bred in rural areas. They continue to be productive under all kinds of difficult conditions and probably adapt better than other gene sources to scenarios that are to be developed under the negative effects of changing climatic conditions. However, breeding of Hair goat in Türkiye is mostly carried out by small family farms under extensive conditions to meet the needs of the family. Therefore, it is obvious that the genotype of the Hair goat breeding under farmer conditions does not reflect its yield potential. For this reason, it is a well-known fact that the genotype of the Hair goat bred by the public cannot reflect its productivity potential and breeders suffer economic losses due to productivity losses in production. In this context, the projects initiated throughout Türkiye aimed to increase the reproductive traits of the local Hair goats breeding in extensive systems under farmers' conditions. Breeders with this study were first trained to keep records and taught how to make yield-oriented breeding selections based on the records. By training the breeders in this way, it has been determined that the reproductive criteria of the breeding animals and performance data such as birth weight, weaning weight, daily body weight gain and survival rate of the kids have improved compared to the first year of production. This study has shown that training farmers producing in extensive system supports the sustainable structure and profitability of production by minimizing possible misapplications during production.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

There is no conflict of interest among the authors.

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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Usability of Jerusalem artichoke tuber waste in lettuce (*Lactuca sativa* var. longifolia) seedling production

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Abstract

The growing medium, which is crucial for seedling production, directly affects germination and the subsequent root system. This medium not only provides support to the plant, but also acts as a source for water and nutrients. It also allows diffusion in the roots and provides gas exchange between the root and the atmosphere outside the root. In this study, the usability of Jerusalem artichoke tuber residues in lettuce seedling cultivation was investigated under greenhouse conditions.For this purpose, was added main media (peat, perlite, peat 50%+perlite 50%) and different proportions Jerusalem artichoke residues of 32, 48, and 96 grams. At the end of the experiment, the number of leaves, plant weight, plant height, leaf length, leaf width, leaf surface area, root length and root weight values were measured. A statistically significant effect of media types was determined in all measured parameters. A statistically significant effect of medium types was determined in all measured parameters. In general, it has been observed that the 32 g L^{-1} Jerusalem artichoke residue additives made to the peat cause an increase in many values. The most prominent issue in increases is that the effect on the values from the weight unit is higher than the ones in the length unit. This result shows that Jerusalem artichoke residues lead to stronger tissues in lettuce seedlings.

Keywords: Growing Media, Organic, Reuse, Roots

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INTRODUCTION

Despite the rapid increase in the world population, the gradual decrease of the limited agricultural areas has increased the need for food and directed the producers to the cultivation of more productive, high quality and disease and pest resistant products from the unit area (Demirsoy &Uzun, 2019).

One of the most important issues affecting the success in vegetable growing is the use of the right seedlings as starting material (Polat et al., 2017). Obtaining quality seedlings is related to both the use of quality seeds and the preparation of the right seedling growing environment. There is a strong relationship between the amount / availability of nutrients and the amount of organic matter in plant growing medium (Demiral, 2000).

A suitable growing environment is essential for quality seedling production and directly affects growth and the subsequent root system. The growth medium is not only the place where the growth takes place, but also serves as a nutrient source for plant growth (Aytekin *et al*., 2021). Good selection of organic and inorganic growing media plays a key role in successful seedling production. It is reported that the growing media mixtures (silt, leaf compost, farm manure, cocopeat and perlite) used in different ratios have significant effects on the physiological and morphological characteristics of the plants (Hussain *et al*., 2016). The quality of the seedlings used is directly related to the yield and quality of the final product. Before that, the environments used in seedling production directly affect seedling development and production cost (Yılmaz & Kınay, 2016).

Peat has traditionally been used as the main seedling growing media component in Europe (Landis *et al*, 2009). Today, annual peat production in Europe is over 40 million m3. high cation exchange capacity, low nutrient levels, low pH, suitable water holding capacity and air capacity are the most important reasons why Peat is in such demand

(Prasad et al, 2018). Despite all these positive features, the high cost of energy used to extract peat and transport it over long distances (which has to be transported from northern Europe to other regions) and its contribution to the carbon footprint raises environmental concerns (Landis et al., 2009, Gruda, 2019).

Studies investigating commercial soilless mixture materials that can be used in seedling growing environments have mostly focused on the availability of regional organic wastes rather than inorganic materials (Polat *et al*., 2017). In addition, some industrial and agricultural waste materials, which cause environmental pollution and financial losses, contribute to the increase of organic matter content of the soil by being evaluated both in seed sowing and seedling planting, and by adding a certain amount of plant nutrients if necessary. A practical solution to minimizing the generation and disposal of waste materials would be to compost the biodegradable waste in situ and then use the compost as a component of the growing medium for seedling production (Veijalainen *et al*., 2008).

Coconut fibers were mixed with peat in different ratios and tried as an organic seedling growing medium in curly lettuce (Colla *et al*., 2007).It has been reported that the highest results in terms of fresh weight, dry weight, and leaf area characteristics of the seedlings were measured in media consisting of 40% and 60% coconut fiber. Aklıbaşında *et al*. (2011) investigated the effects of paddy husk, tuff and peat materials on yellow pine seedling production. At the end of the experiment, the best seedling growth porosity was obtained from peat medium with a porosity of 60.1% and a usable water volume of 15.9% and 10% paddy husk. It was observed that the seedlings did not develop in the environment consisting only of rice husk. Peat with 30% with rice husk medium gave almost the same results as the medium consisting only of peat.

The American origin Helianthus genus has around 50 species grown in the world. Jerusalem artichoke is categorized in this genus (Heiser, 1978). Jerusalem artichoke is highly tolerant of most environmental stress conditions and has the potential to grow aggressively. The most important advantage of rapid growth is the low pesticide requirement and the increased amount of biomass per area. In addition to containing different kinds of vitamins, minerals, it contains complex carbohydrate inulin, which can improve health in humans. Because of these properties, Jerusalem artichoke is considered not only as a human or animal feed, but also as a promising plant for ethanol production (Kays *et al.,* 2007). Although Turkey is not the homeland of Jerusalem artichoke, it has been cultivated for a long time. Considering Turkey's very different ecological conditions and selection by breeders over time, it is assumed that significant genetic variation can be observed in local Jerusalem artichoke accessions. In 2018, the gene pool of Jerusalem Artichokes started to be created at Erciyes University. Within the scope of this project, a collection was created with tuber samples from different provinces and towns of Turkey and this collection was enlarged with crossbreeding studies (Hanci & Tuncer, 2019; Hanci, 2021).

In this study, the usability of dry tuber residues, which occur during the production of Jerusalem artichoke flour, as a medium additive in the production of lettuce seedlings was investigated comparatively.

MATERIALS AND METHODS

The research was carried out in the greenhouses of Sivas-Şarkışla Research Station of Middle Black Sea Transitional Zone Agricultural Research Institute on April 2022. Jerusalem artichoke tuber waste (JA) was obtained from the gene pool of Erciyes University Faculty of Agriculture. In the study, "TS1 Klasmann Deilmann" peat was used as the main component (N:80 mg/dm, P_2O_5: 200 mg/dm, K_2O:360 mg/dm, Mg:100 mg/dm). As the other main component, sterile agricultural perlite was used (pH 6.5-7, 4-6 mm in diameter, with a density of 1 kg/20 lt (±5%)

For the preparation of the media containing Jerusalem artichoke tuber waste, one liter volume of main media (peat, perlite, peat 50%+perlite 50%) were placed in clean containers. Jerusalem artichoke residues are the hard material with a diameter of 0.5-0.7 mm remaining after grinding the tubers dried at 55 degrees Celsius for 36 hours for flour production. (Hanci *et al.* 2020) Lots of 32, 48, and 96 grams of this material were added to one liter of main media and mixed homogeneously.

The prepared mixtures were filled into plastic viols with 4x4x8 cm volumes. Lettuce (*Lactuca sativa* var. longifolia cv. Grise Maraichere) seeds were used as plant material. The seeds were sown at a depth of 1 cm in April 2022. In the experiment, which was carried out in 3 replications according to the randomized plots trial design, 108 seeds were used in each plot.

The experiment was followed for 45 days, the number of leaves, plant weight, plant height, leaf length, leaf width, leaf surface area, root length, root weight were measured in the seedlings obtained and the ratios of the relevant parameters to each other were calculated. The data were analyzed by using JMP Pro 17.0 statistical package program, analysis of variance was performed and statistically significant parameter values according to the results of analysis of variance were compared with LSD test.

RESULTS AND DISCUSSION

As a result of the analysis of variance, it was determined that the difference between the seedling growing media was significant at the 5% level in terms of all lettuce seedling characteristics. The effects of different seedling growing media on seedling characteristics are given in Table 1 and 2. The germination rate of seeds was 100% in the whole experiment.

Table 1. Interaction Effect of Growing Media and Jerusalem Artichoke Supplements.

Main media	Jerusalem Artichoke supplement (g L^{-1})	Number of leaves (pcs)	Whole Plant Weight (g)	Root Weight (g)	Total Leaf Weight (g)	Root/Leaf Ratio (Weight)
Peat	0	16.89±0.4 a	22.67±0.8 bc	4.44±0.1 b	18.22±0.2 bc	0.25±0.02 cd
	32	14.33±0.3 ab	42.11±1.1 a	15.06±0.2 a	27.06±0.5a	0.45±0.08 b
	48	13.44±0.3 bc	33.44±1.0 ab	7.00±0.1 b	26.44±0.6 ab	0.30±0.04 bcd
	96	9.78±0.3 def	20.56±0.9 c	3.72±0.1 b	16.83±0.2 cd	0.21±0.05 cd
Perlite	0	6.67±0.2 f	10.89±0.6 c	1.89±0.1 b	9.00±0.3 d	0.20±0.04 d
	32	8.11±0.3 def	16.33±0.7 c	3.22±0.1 b	13.11±0.4 cd	0.24±0.04 cd
	48	9.56±0.4 def	22.89±0.9 bc	4.72±0.2 b	18.17±0.5 c	0.27±0.05 cd
	96	7.22±0.4 ef	13.78±0.3 c	2.04±0.1 b	11.73±0.4 cd	0.17±0.02 d
Peat+ Perlite	0	10.33±0.4 cde	21.78±0.5 bc	3.7±40.1 b	18.03±0.3 c	0.25±0.04 cd
	32	8.67±0.3 def	23.44±0.5 bc	5.42±0.2 b	18.02±0.4 c	0.30±0.04 bcd
	48	11.22±0.5 bcd	18.67±0.6 c	6.89±0.3 b	11.78±0.2 cd	0.63±0.07 a
	96	9.11±0.4 def	18.89±0.5 c	4.61±0.3 b	14.28±0.4 cd	0.36±0.05 bc
Mean		*10.44±1.6*	*22.12±2.1*	*5.23±1.1*	*16.89±2.2*	*0.30±0.4*
F value		*6.993*	*3.515*	*5.805*	*3.560*	*5.639*

**Means within a column that have a different small letter are significantly different from each other ($p \leq .05$).*

When the number of leaves of the seedlings is examined, it is seen that the highest result is observed in seedlings grown in peat media without JA added (16.89 per plant). The lowest value was obtained from seedlings grown in perlite without any organic material such as peat or JA (6.67 per plant). In whole plant weight values, the addition of 32 and 48 g L^{-1} JA to peat caused higher results than other treatments (42.11 and 33.44 g respectively). As with the number of leaves, perlite media caused these values to decrease, but 48 g L^{-1} JA application increased the values relatively. A difference was observed in the increase in all plant biomass (root+stem+leaf) on peat-based media. This situation has also been experienced in peat+perlite-based media. Although the lowest plant biomass was measured in perlite medium, one of the highest results could be obtained with the addition of 48 g L^{-1} JA (Table 2).

When the whole plant weight and whole plant height values are examined together, it is clearly seen that 32 and 48 g L^{-1} JA additions to peat cause weight gain without affecting the length. This proves that the addition of JA promotes firmer structure of plant tissues. Except for these two treatments, there is a correlation between the two parameters in all results (Figure 1). As a result of the measurement made after the root zone of the plants was removed, the highest value was obtained with the addition of 32 g L^{-1} JA to the peat. The same application gave the highest result in terms of total leaf surface area. While each JA dose increase in peat led to a decrease in the amount of leaves, the weight increase experienced with the addition of 32 and 48 g L^{-1} JA was found to be significant, just as in the root length/weight balance (Figure 2). All seedlings medias examined in the study had the same effect on root weight, except peat+32 g L^{-1} JA (15.06 g). Similar results were also observed in root length results. As with other parameters, the negative effect of perlite was not observed in root weight, but it was repeated in root length. Supplementation of 48 g L^{-1} JA was able to partially eliminate this effect of perlite (Figure 3). The addition of Jerusalem artichoke residues at the highest dose resulted in decreases in all parameters, except total leaf surface area and total leaf weight, compared to the 48 g/l treatment, and in some cases even compared to the control, although this decrease varied depending on the base media.

Table 2. Interaction Effect of Growing Media and Jerusalem Artichoke Supplements.

Main media	Jerusalem Artichoke supplement (g L^{-1})	Whole Plant Length (cm)	Root Length (cm)	Leaf Length (cm)	Leaf Width (cm)	Total Leaf Area (cm^2)	Root/Leaf Ratio (Length)
Peat	0	24.00±0.9 a	3.56±0.4 ab	15.02±0.9 a	3.8±0.4 bc	148.16±3.4 bc	0.24±0.01 bcd
	32	24.11±0.8 a	4.44±0.5 a	11.55 bcd	3.96±0.5 bc	219.98±2.5 a	0.39±0.02 a
	48	24.00±0.9 a	3.33±0.4 b	10.51±0.9 d	3.89±0.6 bc	215.02±3.6 ab	0.32±0.02 ab
	96	23.17±0.7 a	2.33±0.2 cde	12.49 bc	3.79±0.4 bcd	136.87±1.9 cd	0.21±0.03 cd
Perlite	0	16.70±0.6 c	1.74±0.3 de	7.37±0.4 e	3.04±0.4 e	73.18±1.4 d	0.24±0.02 bcd
	32	17.44±0.8 c	1.97±0.2 de	7.38±0.5 e	3.15±0.3 de	106.61±2.0 cd	0.27±0.06 bc
	48	22.50±0.9 a	3.00±0.4 bc	11.69±0.7 bcd	4.19±0.5 abc	147.71±2.3 c	0.27±0.05 bc
	96	17.67±0.5 bc	1.56±0.2 e	10.76±0.5 cd	4.43±0.6 ab	95.40±1.2 cd	0.15±0.02 d
Peat + Perlite	0	23.2±0.9 a	2.59±0.4 bcd	10.45±0.6 d	3.67±0.4 cde	146.63±3.0 c	0.27±0.05 bc
	32	23.00±0.8 a	3.08±0.2 bc	11.99±0.9 bcd	4.13±0.5 abc	146.54±3.1 c	0.27±0.04 bc
	48	22.67±0.9 a	3.56±0.4 ab	12.73±0.9 b	4.64±0.4 a	95.76±2.2 cd	0.28±0.07 bc
	96	21.33±0.8 b	2.72±0.4 bcd	11.48±0.8 bcd	4.08±0.6 abc	116.09±2.1 cd	0.24±0.05 bcd
Mean		*21.66±1.9*	*2.82±0.9*	*11.12±1.1*	*3.90±0.8*	*137.33±2.8*	*0.26±0.09*
F value		*4.168*	*3.012*	*9.778*	*3.979*	*3.560*	*3.032*

**Means within a column that have a different small letter are significantly different from each other ($p \leq .05$).*

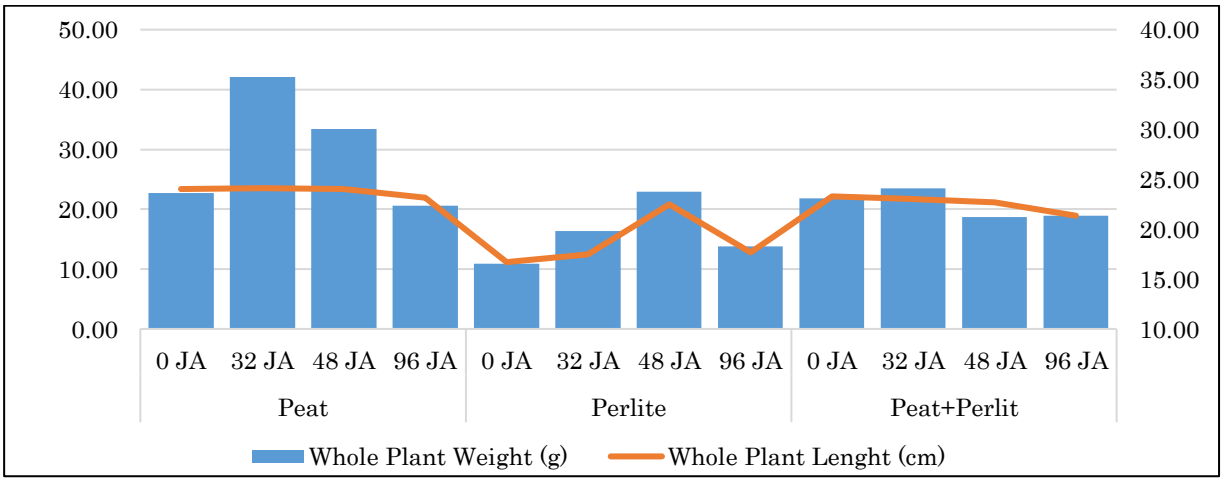

Figure 1. Relationship Between Whole Plant Weight and Whole Plant Length.

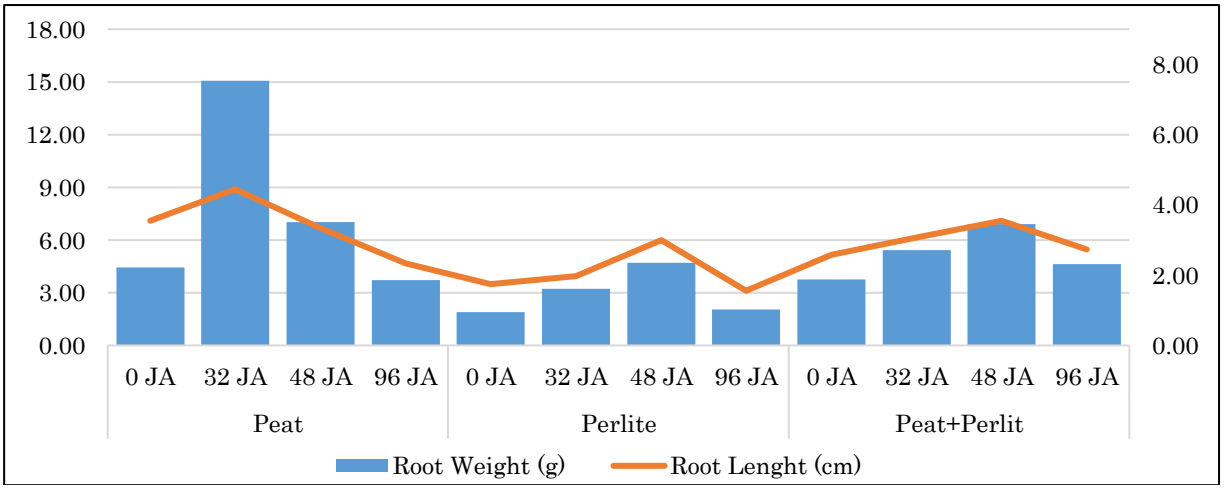

Figure 2. Relationship between root weight and root length.

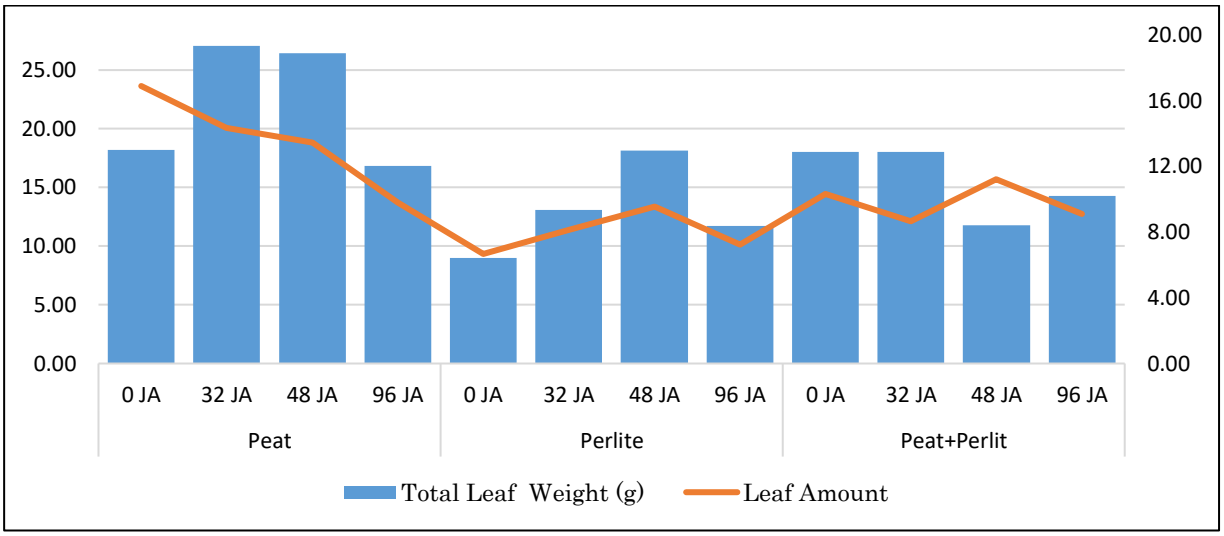

Figure 3. Relationship Between Total Leaf Weight and Number of leaves.

Depending on where they come from, agricultural 174 wastes are not only biodegradable by nature but also full of nutrients (carbohydrates, proteins, fibers, minerals, vitamins, etc.). Waste evaluation not only resolves the issue of disposal but also the issue of environmental degradation. Interest in converting agricultural and industrial wastes into commercially valuable products is growing rapidly. Effective management of agro-industrial waste based on sustainable criteria is an inexpensive, accessible, and widely available alternative that provides a model for adding economic value to the agro-industrial chains of crops (Gruda, 2019).

In the study of Zhu *et al* (2021), residues of Jerusalem artichoke were added in three soils differing in salinity, and the physical and chemical properties of soil and soil microbial community were defined over time. After residue incorporation, the soil organic matter content increased at first and then decreased whereas the soil salinity decreased significantly. pH level fluctuated between 6.8 and 8.0. The population of moderate halophiles and cellulose-decomposing bacteria in soil increased. Overall, the addition of Jerusalem artichoke residues can improve the soil's physical and chemical properties and the microbial community structure. The authors suggest that this situation may ensure a hypothetical foundation for the development of saline-alkali soils, reasonable usage of crop residues, and promoting sustainable development of modern agriculture. Shiven *et al* (2013) investigated the effect of Jerusalem artichoke residues on soil enzyme activities and microbial communities in tomato continuous cropping soil. They used the 2% (w/w) Jerusalem artichoke residue as the treatment material in the soil. At the end of the study, they reported that Jerusalem artichoke residue increased the activity of soil sucrose and urease. In addition, the soil sucrose activity showed a robust negative correlation with Fusarium and Bacillus. In general, the results disclosed that Jerusalem artichoke residues altered the soil bacterial and fungal community compound and the soil enzyme activities. Although the effects of Jerusalem artichoke residues on soil chemistry and biological content were investigated in these studies, the results indirectly support the results of our study. In our study, even at certain doses, Jerusalem artichoke residues positively affected some vegetative parameters of

lettuce seedlings. Especially the addition of 32 g L^{-1} Jerusalem artichoke residue to peat led to significant increases in leaf and root weights

The findings of our study show that the highest dose (96 g L^{-1}) of Jerusalem artichoke residues had a negative effect on plant growth.Some reports indicate that high carbohydrate accumulation in soil can indirectly negatively affect plant growth. In particular, carbohydrates lead to rapid multiplication of microorganisms, causing them to consume more resources essential for plant growth, such as oxygen and nitrogen. This reduces the amount of oxygen reaching plant roots and can lead to depletion of plant nutrients. When microorganisms multiply in soils high in carbohydrates, they start to use more nitrogen for growth and energy production. This can reduce the level of nitrogen needed for plants, which slows down their growth.Carbohydrates, especially when they cause intense microorganism activity, can reduce the oxygen level in the root zone, limiting oxygen uptake by the roots. This can negatively affect plant health, leading to root rot and even plant death (Wang *et al*., 2024; Vincze *et al*., 2024).

Atiyeh *et al.* (2000) compared a standard commercial hydroponic growing medium with alternative media prepared with various organic products. At the end of the study, the germination rates of tomato, pepper, lettuce, and marigold seeds in the coconut/perlite mixture were similar to the results in the standard medium. However, the germination rate of tomato, pepper, and lettuce seedlings was low in peat/perlite medium. Replacing of 10% or 20% of either vermicomposting with coconut/perlite and peat/perlite blends significantly increased seedling growth and overall plant growth was as good as, and sometimes better than the Metro-Mix 360. In the study of Pinter *et al* (2019), the efficiency of the different types of composts (grape marc and a mixture of grape marc, goat manure, and leaves of alfalfa), on the germination and biomass of lettuce seedlings was surveyed. Results showed that composts increased lettuce biomass, with the highest values obtained in mixture compost treatments. Also, the compost mixture indicated the highest seedling biomass. Bassaco *et al.,* (2019) evaluated the effect of rice husk and rabbit manure vermicompost and the bovine rumen on the quality of lettuce seedlings. It was reported that the use of the rice husk and the vermiculite in the substrate improves physical conditions, as a soil conditioner, favoring the lettuce seedling development. The results obtained from these studies are generally in line with the results obtained from our study. In our study, it has been proven again that perlite alone cannot be an ideal growing medium, and the addition of Jerusalem artichokes has relatively eliminated this negativity. The main effective applications are additions to peat. Especially, 32 g L^{-1} of Jerusalem artichoke residue per liter of peat led to an increase in many parameters.

Carbohydrates are used in plant cells for growth and as a source of energy. However, these carbohydrates are mostly stored for cell wall synthesis and as structural components. Cell growth usually occurs through cell expansion and volume increase, which leads to an increase in the biomass of the plant. However, this effect is not directly reflected in length, as length growth is more sensitive to hormonal signals and water balance. Weight gain is a direct consequence of the accumulation of carbohydrates and other components in the tissues (Wang *et al*., 2024; Vincze *et al*., 2024).

In plants, growth in height is mainly controlled by hormones (e.g. gibberellins) and is related to the tendency of cells to elongate. Carbohydrates are concentrated in tissues such as roots, stems or leaves, increasing the weight of the cells. This leads to an increase in biomass in the internal tissues of the plant, with a limited effect on growth in height. In other words, carbohydrates are more associated with the condensation and weighting of tissues, leading to mass growth rather than height growth (Hayat *et al.*, 2010).

CONCLUSION

Reusing organic wastes from plant cultivation or processing in agricultural production and incorporation into growth media can efficiently increase soil organic matter and mineral nutrient content, develop soil structure and fertility, and increase yields. In this study, the combinations formed by the addition of tuber residues from Jerusalem artichoke flour production to various seedling growing media without any pretreatment were compared in the production of lettuce seedlings at the first time. All seedlings medias examined in the study had the same effect on root weight, except peat+32 g L^{-1} JA (15.06 g). Similar results were also observed in root length results. As with other parameters, the negative effect of perlite was not observed in root weight, but it was repeated in root length. Supplementation of 48 g L^{-1} JA was able to partially eliminate this effect of perlite. The results of the study showed that the Jerusalem artichoke residues added to the peat at a rate of 32 g L^{-1} led to an increase in many measured morphological data. The most prominent issue in these increases is that the effect on the values from the weight unit is higher than the ones in the length unit. This result shows that Jerusalem artichoke residues lead to stronger tissues in lettuce seedlings.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

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.

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The efficacy of *Serratia nematodiphila* and Neem Azal T/S on *Macrosiphum rosae*: new approaches in biological control

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Abstract

The rose aphid, *Macrosiphum rosae* (L.) (Hemiptera: Aphididae), is one of the most common pests of rose plants. This study evaluated the effects of four different doses of Neem Azal-T/S, containing the active ingredient Azadirachtin A, and a dose of *Serratia nematodiphila* ($1x10^8$ cfu/ml) on *M. rosae* over 72 hours. The experiment was conducted in a climate chamber under controlled conditions (25±1 °C, 60±5% relative humidity, and a 16:8 light-dark photoperiod). The results showed that Neem Azal-T/S led to mortality rates of 12.5%, 17.5%, 60%, and 77.5%, respectively, while *S. nematodiphila* resulted in a 78% mortality rate after 72 hours. In the control group, mortality was 0.75%, while mortality rates for the treatment groups were 1.25 (Neem_1), 1.75 (Neem_2), 6.00 (Neem_3), 7.00 (Neem_4), and 8.25 (*S. nematodiphila*). Statistical analyses showed significant differences between all treatment groups and the control. In conclusion, this study demonstrated that both Neem Azal-T/S and *S. nematodiphila* significantly increased mortality rates in *M. rosae* compared with the control. Additionally, this study is the first record of the presence of *S. nematodiphila* in Türkiye and the first information on its entomopathogenic effect on *M. rosae* in the worldwide, supporting the potential of biological methods in managing rose aphids and emphasizing the importance of biological control strategies in agricultural pest management.

Keywords: Biocontrol, Entomopathogenic bacteria, Natural insecticides, Insecticidal activity

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INTRODUCTION

Aphids (Hemiptera: Aphididae) are among the most challenging pests to control, causing damage to commercial crops and agricultural food products worldwide (Li et al., 2023). In addition to causing direct harm, aphids also cause indirect damage by transmitting pathogenic plant viruses (Yang et al., 2023). The rose aphid *Macrosiphum rosae* (L.) is a significant pest, particularly in roses, where it feeds on the sap of young leaves, shoots, flower stems, and buds, causing direct damage to the plants (Golizadeh et al., 2017). High aphid populations can lead to serious damage, such as bending of plant stems, weakening of leaves, and premature leaf drop. Moreover, the "honeydew" secreted by aphids promotes sooty mold growth on flowers and leaf surfaces, thereby reducing the aesthetic and commercial value of the plant.

Chemical insecticides are commonly used to control aphid populations. However, excessive and improper use of these pesticides poses risks to both human health and the environment (Alengebawy et al., 2021). Additionally, aphids can develop high resistance to these insecticides over time (Jiang et al., 2018). In recent years, these issues have led to the increased use of botanical insecticides as alternatives to chemical pesticides for pest management (Ngegba et al., 2022). Several studies have demonstrated that plant-derived essential oils derived from plants are effective against aphids (Stankovic et al., 2020). Moreover, there is a growing global interest in developing bio-products as new alternatives to conventional chemical insecticides for controlling pest insects. In this regard, bacteria have emerged as an effective strategy in biological control, offering environmentally friendly solutions.

Bacteria like *Bacillus thuringiensis* produce endotoxins that kill pests by disrupting their digestive systems (Bel et al., 2020). Several bacterial species, including *Burkholderia, Chromobacterium, Pseudomonas, Serratia, Streptomyces*, and *Yersinia*, have been reported to exhibit pathogenic effects on pests (Sarkhandia et al., 2023).

Bacteria of the genus *Serratia* are rod-shaped, gram-negative, and facultative anaerobic bacterium, belongs to the family Enterobacteriaceae (Hejazi & Falkiner, 1997). These bacteria are found in water, soil, plants, insects, humans, and animals (Manzano-Marín et al., 2012). *Serratia* can be distinguished from other genera by producing three specific enzymes: DNase, lipase, and gelatinase (Giri et al., 2004). It is often considered an opportunistic or facultative pathogen since it is frequently avirulent against insects when present in the digestive system, and only becomes lethal when it penetrates the intestinal walls and enters the hemocoel (Aggarwal et al. 2017). The effectiveness of *S. marcescens* against harmful insects is likely associated with the various biologically active compounds produced by the bacteria, including prodigiosin, serrawettin, and several proteases. Prodigiosin is a pigment with antibacterial and antifungal properties that can suppress the immune systems of insect pests, leading to their mortality. Additionally, *S. marcescens* is one of the most effective bacteria for chitin degradation (Monreal & Reese, 1969). When cultured in the presence of chitin, various chitinolytic enzymes and chitin-binding proteins are detected (Suzuki et al., 1998). *S. marcescens* produces at least three chitinases (ChiA, ChiB, and ChiC), one chitinase, and a presumed chitin-binding protein (CBP21) (Cheng & Haas, 1990). Recent reports have highlighted that *Serratia* spp. can cause mortality in insects due to their pathogenic properties. For instance, *S. marcescens* is an entomopathogenic bacterium that causes bacteremia in the hemolymph of insects, leading to rapid death (Lee & Lee, 2022). This bacterium has demonstrated larvicidal effects against Anopheles and Aedes mosquito species (Steven et al., 2021). Similarly, *S. nematodiphila* has been reported to have significant negative effects on the growth and development of the pest *Mythimna separata* (Lin et al., 2024).

Entomopathogens are microbial organisms that cause diseases in insects and are frequently used as biopesticides to control insect pests in various cropping systems (Niu et al., 2022). In our study, we focus on *S. nematodiphila*, another entomopathogenic species, which has shown potential as a biological control agent against various pests. This bacterium suppresses the immune system of insects, rapidly proliferates in the hemolymph, and leads to the pest's death. Studies on the pathogenic effects of *S. nematodiphila* in insects suggest that this bacterium is a promising candidate for controlling pest populations. For example, *S. nematodiphila* (*Serratia* SV6) has shown larvicidal activity against three different mosquito species, with the highest efficacy observed against *Culex quinquefasciatus* (100% after 48 hours), followed by *Anopheles stephensi* (95%) and *Aedes aegypti* (91%) (Patil et al., 2012). Globally, there are limited studies on the effects of *S. nematodiphila* on pest insects (Jackson et al., 2001; Nuñez-Valdez et al., 2008; Patil et al., 2012), and in Türkiye, the identification of this bacterial species represents a significant first record in the scientific literature. This finding has the potential to open new avenues for biological control strategies, particularly in the management of local pests.

In this study, we aim to evaluate the lethal effects of the botanical insecticide Neem Azal T/S and the bacterium *S. nematodiphila* on *M. rosae* nymphs under laboratory conditions. This research seeks to highlight the potential of natural and effective solutions in plant protection practices.

MATERIALS AND METHODS

Collection of rose aphids

The primary colonies of *Macrosiphum rosae* were collected in April 2024 from untreated rose plants in a garden located in Siirt Merkez (37°57′6″N, 41°53′28″E). Infested shoots, buds, and leaves were pruned with shears and immediately transported to the laboratory in a cool environment. The aphids were identified by Associate Professor Işıl Özdemir.

Microorganism isolation

The bacterial isolate was obtained from a wheat plant in Eruh district, Siirt province, in the Southeastern Anatolia region of Türkiye (37°7975120″N, 42°1732090″E). Pure cultures were stored at -20°C using 20% NGB (Nutrient Glycerol Broth). The phenotypic characterization of the bacterium was performed according to (NW, 2001).

Molecular characterization of the microorganism

Genomic DNA of the bacterium was isolated using the Thermo Scientific GeneJET DNA Purification Kit. The 16S rRNA region was amplified using universal primers 27F/1492R (Forward: AGA GTT TGA TCM TGG CTC AG, Reverse: GGT TAC CTT GTT ACG ACT T) following the protocol proposed by Jiang et al. (2006) (95°C for 5 min, [94°C for 30 s, 55°C for 30 s, 72°C for 2 min, 35 cycles], followed by 72°C for 7 min and storage at 4°C). The PCR products were run on 1% agarose gel prepared with TAE buffer (Fermentas, 0.5M) at 80V for 1 hour, and the results were evaluated using a UV transilluminator, with the presence of bands at 1200 bp. The PCR products were sent to MedSantek for bidirectional DNA sequencing analysis. The obtained nucleotide sequences were assembled into contigs, and species identification was performed using the NCBI BLAST database. Subsequently, reference sequences from different *Serratia* species were retrieved from the NCBI database, and a phylogenetic analysis was conducted using the Maximum Likelihood method with 1000 bootstrap replicates in MEGA X software.

Laboratory bioassays

In this study, the commercial product Neem AZAL T/S and the endophytic bacterium *Serratia nematodiphila* isolated from wheat plants were used (Table 1).

Table 1. Bacterial and Neem Azal-T/S doses used in the study

Product	Active Ingredient	Active Ingredient Ratio	Application Dose (ml/100L)
Bacterium	*Serratia nematodiphila*	Pure culture	$1x10^8$ cfu/ml
Neem AZAL T/S	Azadirachtin A	10g/l	500 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	300 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	150 ml/100L
Neem AZAL T/S	Azadirachtin A	10g/l	100 ml/100L

Nymphal individuals of *M. rosae* were used in the study. To avoid damage, nymphs were carefully transferred from infested rose shoots, buds, and leaves using a binocular microscope and placed in petri dishes covered with breathable mesh. Each petri dish (60 mm) was lined with moistened cotton and a composite rose leaf, and 10 aphid nymphs were placed in each dish for each treatment. The trial included the doses specified in Table 1. For the control treatment, only distilled water was sprayed. The experiment was replicated four times. The tests were conducted in a climate chamber set to 25 ± 1°C, 60 ± 5% relative humidity, and a 16:8 (light) photoperiod. The number of live and dead aphids in the petri dishes was recorded 24, 48, and 72 hours after the treatments.

Statistical analysis

Mortality rates were calculated using (Abbott, 1925) formula (Equation 1).

$$\text{Percentage of mortality (M)} = \frac{\text{Live in control (\%)} - \text{Live in treatment (\%)}}{\text{Live in control (\%)}} x100 \quad (1)$$

The data were analyzed using one-way analysis of variance (ANOVA) followed by the LSD test for multiple comparisons at a 95% confidence level. The "Agricolae" package in the R statistical software was used for statistical analysis (de Mendiburu & de Mendiburu, 2019) Probit analyses of Neem Azal T/S were conducted using the Probit MSChart (2024) package program (Chi 2024), providing a detailed assessment of the insecticide's effectiveness on *M. rosae* nymphs.

RESULTS

In this study, the lethal effects of Neem Azal-T/S and the bacterium *Serratia nematodiphila* on *Macrosiphum rosae* were examined. Four different doses of the biological insecticide Neem Azal-T/S, whose active ingredient is Azadirachtin A derived naturally from the neem tree, showed mortality rates of 12.5%, 17.5%, 60%, and 77.5% after 72 hours compared to the control. Additionally, when the effects of *S. nematodiphila* at a dose of $1x10^8$ cfu/ml on *M. rosae* were evaluated, the mortality rate was found to be 78% after 72 hours.

The results demonstrated that the first two doses of Neem Azal-T/S had a very low lethal effect on *M. rosae* and were not statistically significant compared to the control group. However, the 3rd and 4th doses, as well as the *S. nematodiphila* group, showed statistically significant differences compared to the control group (Figure 1).

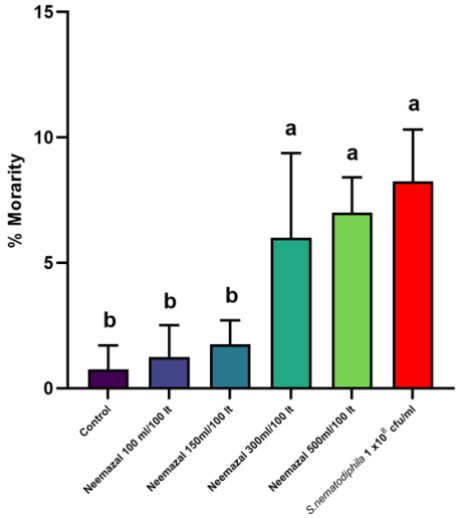

Figure 1. Mortality rates of *Macrosiphum rosae* nymphs in response to different concentrations of Neem Azal-T/S and *Serratia nematodiphila* treatment. Significant differences from the control ($p < 0.05$)

In addition, statistically significant differences were identified between the control group and the various treatment groups (Neem_1, Neem_2, Neem_3, Neem_4, *S. nematodiphila*) (Table 2). Notably, the Neem_3, Neem_4, and *S. nematodiphila* groups were observed to be significantly different from the control group (Table 2).

Table 2. Analysis of variance of the effects of treatment groups on *Macrosiphum rosae*

	Df	Sum Sq	Mean Sg	F value	P (>F)
Treatment	5	216.33	43.3	12.36	2.651×10^{-5}***
Residuals	18	63.00	3.5		

Based on the data obtained from the study, the mortality rate of the control group was 0.75, while the mortality rates for the other treatment groups were 1.25 (Neem_1), 1.75 (Neem_2), 6.00 (Neem_3), 7.00 (Neem_4), and 8.25 (*S. nematodiphila*). Notably, significant differences among these values were found to be statistically important. Additionally, the "LCL" (Lower Confidence Limit) for Neem_3 was 4.03 and the "UCL" (Upper Confidence Limit) was 7.96, while the "LCL" for the Neem_4 group was 5.03 and the "UCL" was 8.96. In contrast, the control group had an "LCL" of -1.22 and a "UCL" of 2.72. The comparison of these ranges indicates that the Neem_3 and Neem_4 groups were distinctly different from the control group. Furthermore, it was found that the *S. nematodiphila* treatment group exhibited a maximum mortality rate of 10 individuals, which was statistically different from the control group (2 individuals). In conclusion, the findings of this study clearly indicate statistically significant differences between the control group and the other treatment groups (Table 3).

Table 3. Analysis of variance of the effects of treatment groups on *Macrosiphum rosae* mortality

Treatment	Mortality	r	std	se	LCL	UCL	Min	Max	Q25	Q50	Q75
Control	0.75 b	4	0.96	0.94	-1.22	2.72	0	2	0.00	0.5	1.25
Neem_1	1.25 b	4	1.26	0.94	-0.72	3.22	0	3	0.75	1.0	1.50
Neem_2	1.75 b	4	0.96	0.94	-0.22	3.72	1	3	1.00	1.5	2.25
Neem_3	6.00 a	4	3.37	0.94	4.03	7.96	1	8	5.50	7.5	8.0
Neem_4	7.00 a	4	1.41	0.94	5.03	8.96	6	9	6.00	6.5	7.5
Serratia nematophila	8.25 a	4	2.06	0.94	6.28	10.21	6	10	6.75	8.5	10

In this study, the probit analysis of four different doses of Neem Azal demonstrated a dose-dependent effect on *M. rosae* nymphs. The LD50 value indicates the effective dose of the insecticide, with higher doses resulting in increased mortality rates. The fiducial limits reflect the precision and uncertainty of the results. These findings suggest that Neem Azal could be an effective biopesticide option for pest control (Figure 2).

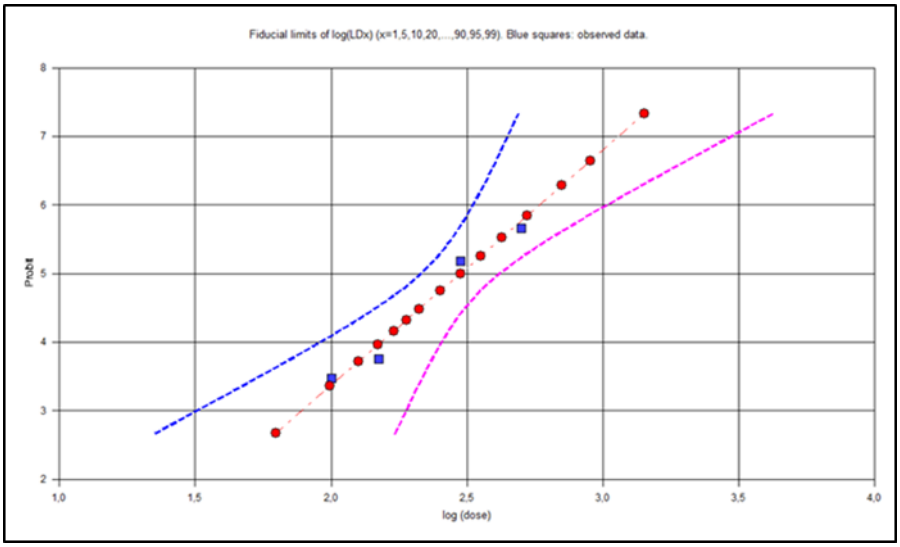

Figure 2. Probit analysis of Neem Azal T/S doses on *Macrosiphum rosae* nymphs

The observed mortality rates of *M. rosae* nymphs in response to different doses of Neem Azal T/S are presented in the graph. The results show a clear dose-dependent relationship between the applied dose and mortality. At lower doses, the mortality rate remains below 10%, while at higher doses (300 units), mortality exceeds 70%. These findings confirm that Neem Azal exhibits a dose-dependent effect and suggest its potential as an effective biopesticide for pest control (Figure 3).

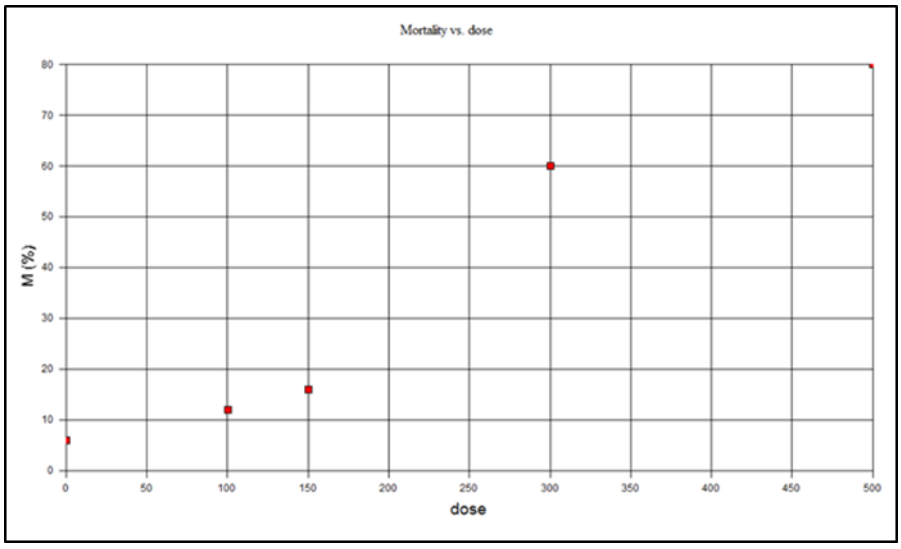

Figure 3. Mortality of *Macrosiphum rosae* nymphs at different doses of Neem Azal T/S

The increasing concentrations of Neem Azal T/S demonstrate a rise in mortality rates among *M. rosae* nymphs. The higher probit values support the effect of increasing doses on mortality. These findings indicate that Neem Azal T/S is an effective biopesticide that exhibits a dose-dependent effect (Table 4).

Table 4. Probit analysis of Neem Azal T/S effects on *Macrosiphum rosae* mortality

Concentrations	N	R	M	probit
0	50	3	6	
100	50	6	12	3,47
150	50	8	16	3,75
300	50	30	60	5,18
500	50	38	76	5,65

The LD50 value of Neem Azal T/S has been determined to be 298.6, and the LD90 value is 705.5. These results are important for evaluating the efficacy and safety of the pesticide. The LD50 value represents the dose at which 50% mortality occurs, and its calculation of 298.6 units indicates that Neem Azal T/S is an effective biopesticide against *M. rosae* nymphs. Furthermore, the LD90 value of 705.5 suggests the potential for the pesticide to achieve 90% mortality at higher doses. These findings demonstrate that Neem Azal T/S is both an effective and practical control agent, capable of being used safely within a specific dosage range in plant protection applications. Therefore, Neem Azal T/S should be considered an important biopesticide option for both agricultural practices and entomological research.

This study also uncovered significant insights into the identity of the isolated strain and its potential contributions to the treatment's effectiveness alongside the promising results obtained with Neem Azal T/S. Additionally, an NCBI analysis revealed that our isolated strain exhibited a 97.76% similarity to *S. nematophila*. Phylogenetic analyses based on reference sequences further confirmed that the isolated bacterium belongs to the *S. nematophila* species, indicating a strong correlation between the identified bacterium and the treatment's efficacy observed in the study. Phylogenetically similar to the closest reference isolate, *S. nematodiphila* DZ0503SBS1 (accesion number: NR 044385.1). It then showed similarity with *Serratia marcescens* KRED NR (accesion number: 036886.1), *Serratia ureilytica* NiVa 51 (accesion number: NR 042356.1) and *Serratia rubidaea* JCM1240 (accesion number: NR 024644.1). Phylogenetically most distant *Serratia glossinae* DUCC3749 (accesion number: KP318496.1) and *Serratia fonticola* DSM 4576 (accesion number: NR 025339.1) (Figure 4).

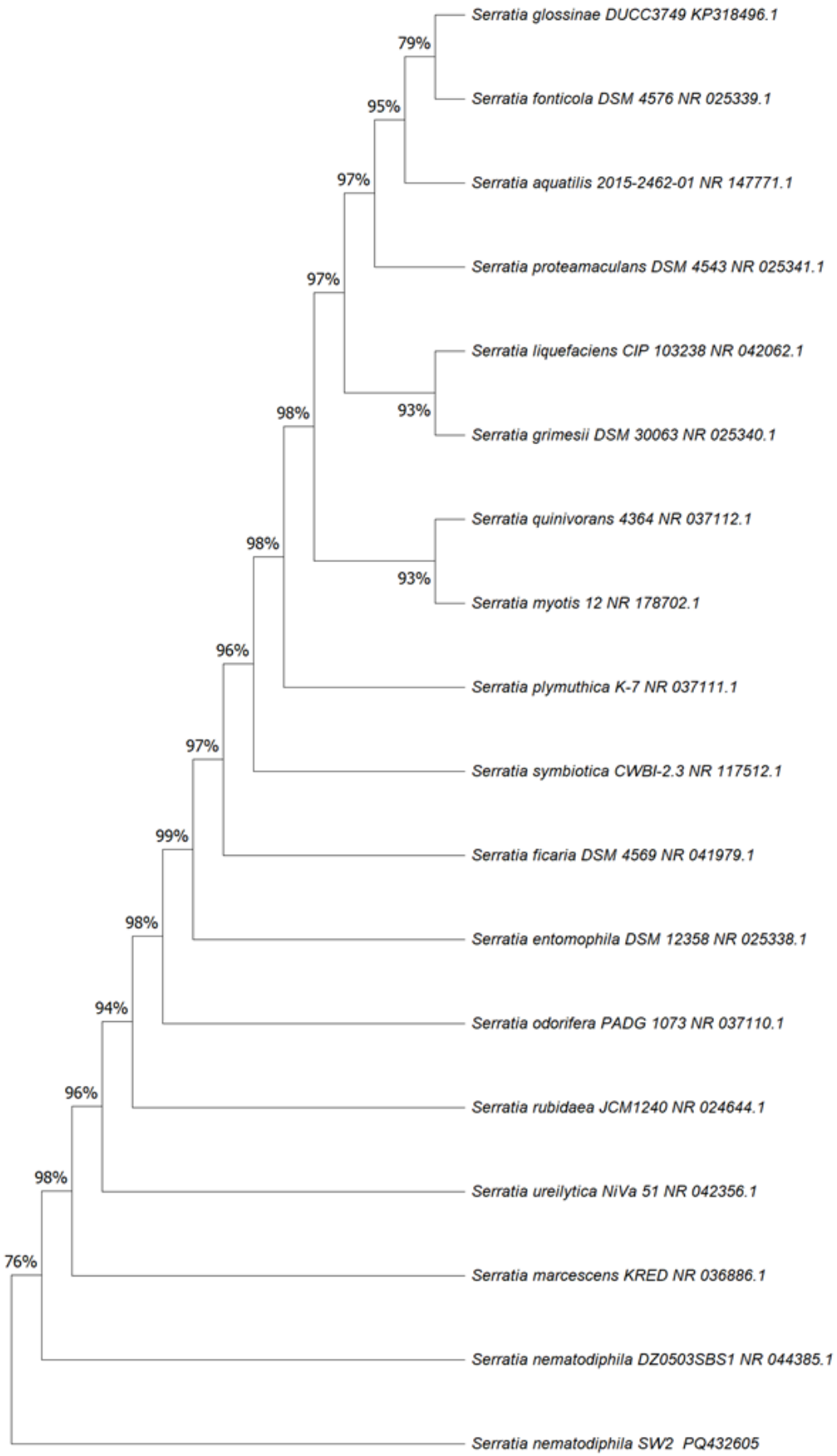

Figure 4. Maximum Likelihood phylogenetic analysis of *Serratia nematophila*

DISCUSSION

Based on the data obtained in our study, the effects of Neem Azal T/S and *S. nematophila* treatment groups on the rose aphid, *M. rosae*, significantly differed compared to the control group. Similar studies in the literature support these findings and present various results regarding the efficacy of Neem and *S. nematophila* against pests. For instance, in our study investigating different doses of Neem, the effects of the Neem_1 and Neem_2 dose groups on pests were found to be relatively low (12.5% and 17.5%). However, in higher doses, such as Neem_3 and Neem_4, this effect increased significantly (60% and 77.5%). Similarly, other studies have reported that Neem applied at higher doses resulted in mortality rates ranging from 60% to 100% in cotton aphids (Santos et al., 2004). Neem-based products have been shown to reduce the colonization of *Myzus persicae* (Sulzer) by 50% to 75% compared to the control group after one week of application (Shannag et al., 2014). In another study, it was found that 1% neem oil reduced the population of *Aphis craccivora* Koch by 74.1% (Mohapatra et al., 2021). Additionally, other literature indicates that Neem extracts caused a 73% reduction in the number of aphids per plant (Muhammad et al., 2018), and a 74.21% reduction in wheat aphids (*Rhopalosiphum padi* (L.) and *R. maidis* (Fitch) (Pathania et al., 2023). These findings indicate that the results of our study are consistent with other research in the literature and demonstrate that Neem Azal-T/S could be a potential option for controlling aphid pests. Indeed, (Bartelsmeier et al., 2022) reported that Neem Azal-T/S applications could be utilized in integrated pest management (IPM) systems to control rose aphids.

On the other hand, the observed 78% reduction in *M. rosae* populations in the group treated with *S. nematodiphila* demonstrates the significant potential of this beneficial bacterium in biological control strategies. This finding is further supported by various reports on the insecticidal potential of *Serratia* species against agricultural pests (Hu et al., 2021; Inglis & Lawrence, 2001; Kim et al., 2009; Konecka et al., 2019; Secil et al., 2012; Wang Lei et al., 2010). However, limited studies have previously investigated the entomopathogenic properties of *S. nematodiphila*. In an earlier study, this bacterial species exhibited the highest efficacy (100%) against *Culex quinquefasciatus* after 48 hours of exposure, followed by *Anopheles stephensi* (95%) and Aedes aegypti (91%) (Patil et al., 2012). Besides, the efficacy of *Serratia* species against various agricultural pests has been demonstrated in previous studies. For example, Wang Lei et al. (2010) reported that *S. marcescens* provided a lethal effect against aphids. Furthermore, Secil et al. (2012) reported mortality rates of *Serratia* sp. On7 (60%) and *S. marcescens* On16 (50%) against *Ostrinia nubilalis* (Lepidoptera: Pyralidae). In another study, *S. marcescens* exhibited insecticidal activity against *Spodoptera exigua* and caused high mortality rates in larvae (Konecka et al., 2019). The 78% mortality rate observed in our study aligns with the results reported in the literature and suggests that *S. nematodiphila* may be effective against a wide range of plant pests.

CONCLUSIONS

This study was conducted to evaluate the potential of the plant-based Neem Azal T/S insecticide and *Serratia nematodiphila* bacteria in the biological control of *Macrosiphum rosae*. The results obtained indicate that both Neem Azal T/S and the beneficial bacterium *S. nematodiphila* are effective against aphids and could serve as alternatives to chemical pesticides. Neem Azal T/S exhibits insecticidal properties due to its active ingredient, Azadirachtin A. This study observed significant mortality rates in aphids with the application of high doses of Neem Azal T/S. However, its efficacy was found to be limited at lower doses, highlighting the importance of appropriate dosage.

On the other hand, *S. nematodiphila* was also evaluated as an effective biological control agent against aphids. The application of a specific dose (e.g., $1x10^8$ cfu/ml) of *S. nematodiphila* resulted in significant reductions in aphid populations. The use of *S. nematodiphila* as a biopesticide offers a sustainable alternative to reduce the negative environmental and health impacts of chemical pesticides. The advantages of bacterial biopesticides include low environmental persistence, minimal harm to non-target organisms, and the potential for preserving biodiversity. However, careful assessment should be conducted regarding the potential effects of *S. nematodiphila* on the ecosystem during its application and the pathogenic characteristics of the bacterium. In particular, the impacts of *S. nematodiphila* on human and animal health should be investigated.

Our findings suggest that this bacterium could be an alternative option for the biological control of pests. However, further research is needed regarding the efficacy of these biological control methods at a commercial scale, their long-term effects, and application strategies. Especially, more detailed studies are required to examine the environmental effects of these agents, their impact on soil microbiomes, and their potential effects on human health. The use of biological pesticides has the potential to reduce the harmful environmental effects of chemical pesticides, but this potential needs to be fully assessed and optimized.

In conclusion, this study represents the first record of the presence of *S. nematodiphila* in Türkiye and provides the first global data on its effects on *M. rosae*. Both the plant-based insecticide Neem Azal T/S and S. nematodiphila bacteria show promise as important options for the biological control of agricultural pests. However, further research is needed to ensure their effective and safe application and to optimize their use in agricultural practices.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

All authors have reviewed and approved the final manuscript. They confirm that the text, figures, and tables are original and have not been previously published.

H. D.: Supervision, conceptualization, methodology, laboratory work, review, and editing

U.Ş: Laboratory work, investigation, bacterial production, and molecular analysis.

M.K: Data evaluation and investigation.

M. D.: Field sampling and data collection.

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Synergist effects of some PGPR bacteria and sodium nitroprusside in pepper plant

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Abstract

Plant growth promoting rhizobacteria (PGPR) represent promotes plant growth by increasing the supply or availability of nutrients to the host plant. These bacterial applications are environmentally friendly techniques and their use has become widespread recently. Some PGPRs can increase nitrogen (N) fixation and have phosphate (P) solubilizing property. In the current study, we evaluated the synergistic effects of some useful bacteria and sodium nitroprusside (SNP, a nitric oxide donor) in pepper plant. Nitric oxide (NO) acts as a signal molecule in plants and has important role in plant-bacteria symbiosis interaction. Three PGPR strains namely, *Enterobacter cloacae* (ZE-2), *Pseudomonas putida* (ZE-12) and *Acinetobacter calcoaceticus* (ZE-13) were used and the bacteria possess phosphorous-solubilizing and nitrogen-fixing properties. The applications of PGPRs alone and with combination of SNP (0.1 mM) were performed to the plant rhizosphere (the roots) through irrigation two times with two weeks interval starting with seedling planting. End of the study, many morphological parameters including stem diameter, plant height and biomass were improved by all applications compared to control. Root:shoot dry weight ratio decreased by the applications. Stem diameter, plant height and biomass were significantly increased with all treatments compared to control. The yield was found higher in all applications compared to control and the highest increase in the yield was provided by *Enterobacter cloacae* (ZE-2) application. Dry matter allocation in upper part of the plants provided higher plant yield. The applications significantly affected cell expansion and division. SNP increased the effect of *Acinetobacter calcoaceticus* (ZE-13) bacteria on cell division in leaf cells and midrib size. Furthermore, *Pseudomonas putida* (ZE-12) increased the yield combining with SNP compared to alone use. The increase in the plant growth is related with the midrib size. The application of PGPR with SNP could be a promising approach in plant growing.

Keywords: Biofertilizer, *Capsicum annuum*, Nitric oxide, PGPR, Yield

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INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which colonize the roots of plants that enhance plant growth (Vessey, 2003). PGPR often called as biofertilizers when applied to seed, plant surfaces, or soil, colonizes the rhizosphere and promotes growth by increasing the supply or availability of nutrients to the host plant (Zaidi et al., 2015). Useful bacteria applications are eco-friendly techniques that conserve the environment against pollution caused by agrochemical products (Tahiri et al., 2022). Many PGPRs have useful effects on plant growth, nutrient availability, and synthesis of hormones (İpek et al., 2017). Some PGPRs can increase nitrogen (N) fixation (Dixon and Kahn, 2004). Phosphate (P) solubilizing property was also reported in many PGPRs (Ergin and Gülser, 2016). Furthermore, some PGPRs are

used against environmental stress factors including salinity (Vaishnav et al., 2016), drought (Admassie et al., 2022) and limey soil (Aras et al., 2018).

A wide variety of bacteria including species of Bacillus, Alcaligenes, Pantoea, Enterobacter have been used for the beneficial effects in many plants (Collavino et al., 2010; Arıkan et al., 2018). The useful effects of Alcaligenes, Agrobacterium, Staphylococcus, Bacillus and Pantoea strains were reported in pear trees under high limey soil conditions (İpek et al., 2017). García et al. (2003) reported that Bacillus and Pseudomonas strains improved tomato and pepper growth. Islam et al. (2013) stated that some N-fixing bacteria increased tomato and pepper growth.

Another way to improve plant growth is nitric oxide (NO) in plants. Among the NO donors, sodium nitroprusside (SNP) is one of the most utilized one (Filippou et al., 2012). Many studies showed that SNP controls stomatal movements, photosynthesis and flowering (Takahashi and Yamasaki 2002; He et al., 2004). SNP triggered early xylem formation in peach bud (Aras, 2022c). SNP application improved cortical cells and xylem vessels in peach leaves (Aras, 2022b). Furthermore, many studies showed that SNP application improved the tolerance against environmental stress factors in many plants (Hayat et al., 2012; Esringu et al., 2016; Kaya et al., 2019; Aras et al., 2020).

NO acts as a signal molecule in plants (Siddiqui et al., 2011). NO possesses important role in plant-bacteria symbiosis interaction (Wang and Ruby, 2011). Vaishnav et al. (2016) reported that NO improved the effects of PGPRs on salinity stress in soybean. A few study were conducted on the interaction between NO and bacteria (Sharma et al., 2021). Thus, in the current experiment we studied the relation between SNP and some useful bacteria including *Enterobacter cloacae*, *Pseudomonas putida* and *Acinetobacter calcoaceticus* in pepper plant. The plant growth, yield and leaf cellular physiology were evaluated in the study with application of PGPRs alone and with combination of SNP.

MATERIALS AND METHODS

Bacterial strains

Within the scope of PGPR acquisition studies, pepper plant growing areas in Tokat province and its districts were visited and samples were taken from the root zone soil of the plant. During sampling, pepper plants that showed better development and appeared healthier than other plants were selected. Bacteria obtained using various isolation methods were purified according to different colony structures. Purified isolates were identified by proteomics at Mustafa Kemal University Plant Health Clinic Application and Research Center using MALDI-TOF MS method. As a result of identification, it was determined that there were three PGPR strains: *Enterobacter cloacae* (ZE-2), *Pseudomonas putida* (ZE-12) and *Acinetobacter calcoaceticus* (ZE-13). In order to determine that the isolates obtained were not plant pathogens, they were subjected to hypersensitivity tests on tobacco plants (*Nicotiana tabacum* cv. Samsun) and softening tests on potato slices. Positive result was not observed in hypersensitive test in tobacco (Belgüzar et al., 2021). None of the isolates was able to cause the death of local cell of tissue in tobacco leaves and rotting of potato slices. Therefore, the bacteria are non-pathogenic.

Bacterial isolates are stored as stock cultures in the Phytopathology laboratory of Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection. King B medium was used for the growth of bacteria. Bacterial suspensions colony densities of bacteria were prepared by measuring the absorbance value of $2x10^{-8}$ (A 600:0.3) using a spectrophotometer with sterile water.

In vitro screening of bacteria properties

The property of the bacteria to solubilize phosphorus (P) was evaluated using National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999). The assay was carried out using a randomized design with three replicates. Three drops of cell suspension were inoculated onto the surface of NBRIP agar plate media, followed by incubation for a week at 25 °C. One colony with the largest clear zone was selected (Johri et al., 1999; Mirik et al., 2008). Nitrogen fixation property of the bacteria was evaluated N-free Jensen agar media (Ahmad et al., 2005).

Pot experiment and bacteria and SNP applications

This study was conducted in a semi-controlled greenhouse (average 25°C temperature, 65% relative humidity) at Yozgat Bozok University, in Turkey. Seedlings of pepper (*Capsicum annuum* L. cv. Çetinel) were planted in 4 L plastic pots filled with substrate and agricultural perlite (4:1). The applications were performed to the plant rhizosphere (the roots) through irrigation two times with two weeks interval starting with seedling planting. Bacterial suspensions were diluted to a final concentration of 10^9 cfu mL^{-1} and applied to the pepper roots. 0.1 mM SNP dose was chosen according to the previous experiments (Kaya et al., 2019; Shams et al., 2019). The applications were performed at the same time. Control plants were not treated with any bacteria or SNP. The study was carried out in a completely randomized design with 3 replicates per treatment and 4 seedlings were used per replicate. The plants were evaluated two months after applications initiation. The yield was determined during the season.

Morphological Responses and Yield

Stem diameter (mm) was measured with a digital caliper. Plant biomass (root+shoot) was determined with a precision balance. Plant height (mm) and root length (mm) were measured with a ruler. Root and shoot dry weights were measured after drying the plant material at 70ºC for 48-72 hours. The value of root:shoot in dry weight was calculated as the dry weights of root/shoot. Yield (g) was determined per plant.

Histological Responses

The samples of the leaves were stored in FAA (formaldehyde, alcohol, acetic acid) solution. Freehand cross sections of leaf midrib were utilized for microscopic evaluation. The midrib sections were subjected to toluidine blue O for cortical cell observation (O'Brien et al., 1964) and visualized with a light microscope (Olympus CX21; Olympus, Tokyo, Japan) coupled to a digital camera.

Statistical Analyses

The statistical analyses were performed with the statistical software package SPSS, version 20.0. Data were subjected to Duncan's test at a significance level of $P < 0.05$. Heatmap and principal component analysis (PCA) was performed using XLSTAT Software.

RESULTS

In the present study, all bacteria had the ability of phosphorous-solubilizing and nitrogen-fixing. The PGPRs alone or combining with SNP improved plant growth and yield in pepper. Stem diameter, plant height and biomass were significantly increased with all treatments compared to control (Table 1). Figure 1 shows the growth increment in pepper provided by the applications.

Figure 1. Pepper plant growth increment provided by PGPRs and SNP

Table 1. Effect of PGPRs and SNP on stem diameter, plant height and biomass in pepper

Treatments	Stem diameter (mm)	Plant height (mm)	Plant biomass (g)
Control	3.97 b	37.6 e	30.0 cd
SNP	4.39 ab	47.0 d	40.3 b
ZE-2	4.60 a	55.1 bc	40.7 b
ZE-12	4.72 a	66.6 a	49.1 a
ZE-13	4.87 a	67.3 a	40.9 b
ZE-2+SNP	4.60 a	60.6 ab	24.7 d
ZE-12+SNP	4.65 a	56.8 bc	35.3 bc
ZE13+SNP	4.63 a	48.6 cd	39.5 b

Root:shoot dry weight ratio decreased by the applications (Table 2). Root length differently affected by the applications. SNP, ZE-2 and ZE-12+SNP increased root length compared to control, when the other applications decreased the value. The yield was remarkably affected by the applications. The highest yield was obtained in ZE-2 application.

Table 2. Effect of PGPRs and SNP on root:shoot in dry weight, root length and yield in pepper

Treatments	Root:shoot in dry weight	Root length (mm)	Yield (g/plant)
Control	0.9056 a	28.5 ab	230 g
SNP	0.5317 b	29.0 a	495 c
ZE-2	0.5526 b	29.5 a	608 a
ZE-12	0.5595 b	25.2 bc	285 f
ZE-13	0.7761 ab	24.0 c	430 e
ZE-2+SNP	0.5654 b	22.0 c	575 b
ZE-12+SNP	0.5666 b	30.5 a	471 d
ZE-13+SNP	0.7854 ab	22.0 c	305 f

We evaluated pepper leaf midrib with histological analyses (Figure 2). The applications significantly affected cell expansion and division (Table 3). The highest cortical cell diameter was found in ZE-2 application that demonstrates the highest increase in cell expansion was found in ZE-2 application. ZE-13+SNP application had the highest number of cortex cell layer among the treatments. In general, the results showed that the treatments did not have a significant effect in cortex cell layer. Moreover, we evaluated midrib diameter in the study. ZE-13+SNP had the highest midrib diameter among the treatments followed by ZE-12+SNP.

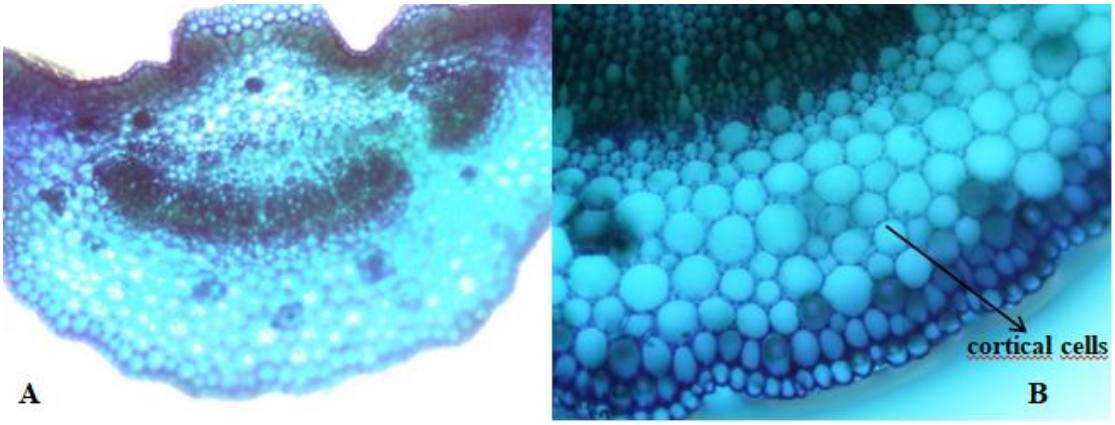

Figure 2. General view of cortical cells of leaf midrib in pepper, A: cross section of leaf midrib, B: cortex of midrib

The normalized heatmap approach was applied to assess all results provided by PGPRs and SNP in pepper (Figure 3). Two main clusters were detected: the first cluster is closely linked to number of cortex cell layer and root:shoot DW, and the second cluster is linked to root length, yield, plant biomass, midrib diameter, cortical cell diameter, stem diameter and plant height. The applications were categorized into two: first is control; and second is SNP, ZE-2+SNP, ZE-2, ZE-12+SNP, ZE-13+SNP, ZE-12 and ZE-13. We also performed PCA to examine the overall distribution trend among applications (Figure 4). Principal Component 1 (PC1) and Principal Component 2 (PC2) were the first and second largest dimensions of data difference and explained the results by 44.13 and 21.79%, respectively. PCA has scattered the applications in four quarters of the biplot. Biplot indicates that PGPRs alone or combining with SNP improved plant growth, histological response and yield compared to control.

Table 3. Effect of PGPRs and SNP on cortical cell diameter, number of cortex cell layer and midrib diameter in pepper

Treatments	Cortical cell diameter (µm)	Number of cortex cell layer	Midrib diameter (µm)
Control	41.3 c	5.54 ab	872 d
SNP	48.2 bc	6.39 ab	927 cd
ZE-2	58.3 a	5.46 ab	1015 bc
ZE-12	54.1 ab	5.22 b	1043 ac
ZE-13	54.6 ab	5.92 ab	1049 ac
ZE-2+SNP	54.1 ab	5.65 ab	990 bd
ZE-12+SNP	56.1 ab	5.45 ab	1106 ab
ZE-13+SNP	52.3 ab	6.61 a	1163 a

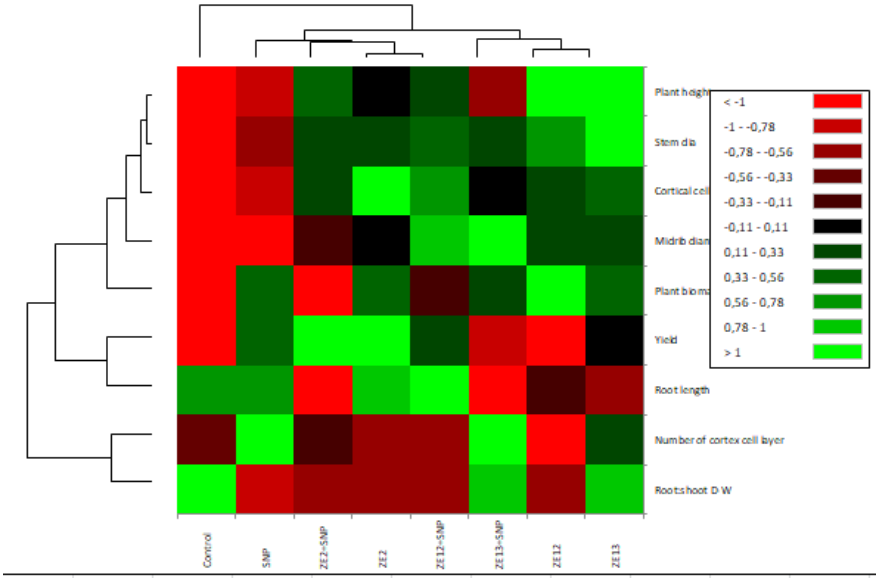

Figure 3. Normalised heatmap responses of the morphological and histological attributes and yield of pepper.

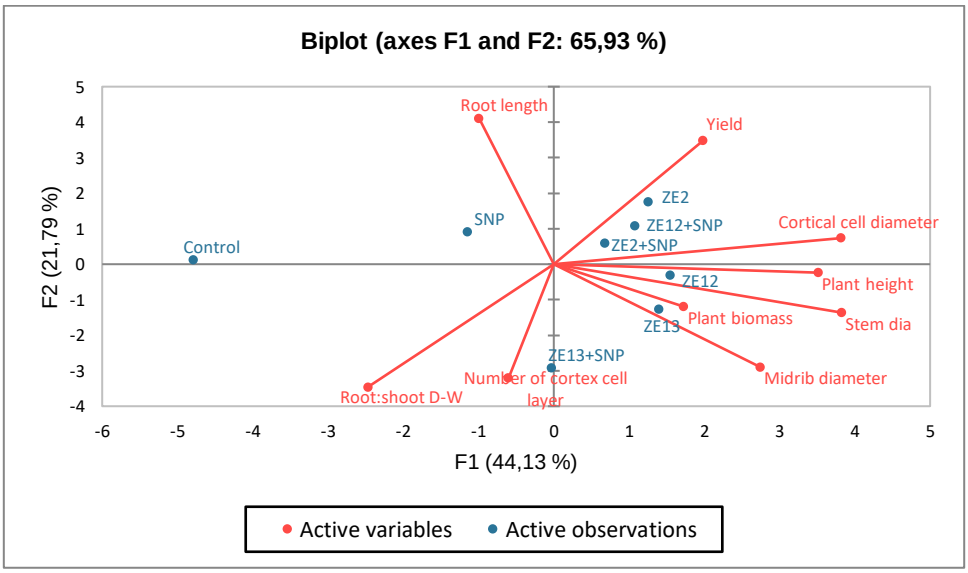

Figure 4. Principal component analysis of the morphological and histological responses and yield of pepper. The mean values of the replicates are shown.

DISCUSSION

The present study demonstrated the effects of some PGPRs alone or combining with SNP in pepper plant. PGPR application possessed a remarkable effect on plant growth and yield.

In this study, which was conducted within the scope of determining the effects of some plant growth promoting rhizobacteria and mycorrhizae on the growth and development of *Dahlia variabilis* (star flower), which is widely produced as cut flower and outdoor ornamental plant in the world and is increasingly widespread in our country, *Enterobacter cloacae* (ZE-2), *Bacillus cereus* (ZE-7), *Pseudomonas putida* (ZE-12), *Acinetobacter calcoaceticus* (ZE-13), *Burkholderia cepecia* (7-a-2) bacterial species and commercially available mycorrhiza (5000 ppm) were applied to the seeds of *D. variabilis* variety 'Violet'. Germination rate (%), seedling height (cm), stem diameter (mm), number of leaves (number), plant fresh weight (g), plant dry weight (g), root length (cm), root fresh weight (g), root dry weight (g) and SPAD value and chlorophyll content were measured. As a result, it was determined that the applications had different effects on D. variabilis. It was determined that *P. putida* (ZE-12) application increased germination by 12% compared to the control, and *A. calcoaceticus* (ZE-13) application increased seedling height by 32.9% (Alkaç et al., 2022a).

In another study, the effects of *Zinnia elegans* L. Zesty and *Dahlia variabilis* L. 'Figaro Violte' on the development of varieties were investigated. *Dahlia variabilis* L. seedlings were planted in suspensions prepared from ZE-12, ZE-13 and ZE-12+ZE13, while *Z.elegans* seedlings were planted in suspensions prepared from ZE-2, ZE-7, ZE-12, ZE-13, ZE-12+ZE-13. Seedlings to which no rhizobacteria and mycorrhiza applications were made were used as the control group. As a result, all parameters except root dry weight of the applications applied in the *D. variabilis* trial remained at the same values as the control. ZE-13 application was effective in root dry weight. It was determined that the applications applied in *Z.elegans* seedlings increased the flower stalk thickness and number of leaves, and especially ZE-13 application was the most effective application. As a result, it was determined that there is a potential for applications that will not negatively affect environmental and human health in ornamental plant cultivation (Alkaç et al.,2022b).

PGPR applications improved plant growth compared to control. The highest increment in stem diameter, plant height and plant biomass was found in PGPR applied plants. PGPR combining with SNP also increased the parameters compared to control. However, the increments were found higher in the use of PGPR alone. Many experiments showed that PGPR application can enhance plant growth in many plants (Mia et al., 2010; Wang et al., 2022). The increase in plant growth is frequently associated with a higher N fixation (Olivera et al., 2004). Aminifard et al. (2012) stated that nitrogenous fertilizer promoted plant growth in pepper. In the current study, we evaluated the effect of phosphorus-solubilizing and N-fixing bacteria on plant growth. The increase in plant growth may be a result of N-fixing property of the bacteria. Bulut (2013) reported that some phosphorus-solubilizing and N-fixing bacteria increased wheat plant growth. Moreover, many N-fixing bacteria enhanced plant growth in many plants (Mohammed et al., 2012; Devi et al., 2022). SNP also increased plant growth both the use of alone and combining with the bacteria. Exogenous SNP application improves plant growth by mediating the level of endogenous NO (Li et al., 2022) and endogenous NO plays important role in photosynthesis (Fatma et al., 2016). In a previous study, we reported that SNP improved chlorophyll biosynthesis and xylem vessel in peach leaves (Aras, 2022b). Xylem is responsible for water and mineral uptake (Brodersen et al., 2019) that may play pivotal role in plant growth.

Moreover, we evaluated root:shoot ratio in dry weight in order to dry matter distribution between shoot and root. All applications decreased the ratio compared to control (Table 2). The decline in root length and increment in plant height were also found in SNP and PGPR applications that provides that SNP, PGPR alone and combining with SNP applications favoured an investment in plant shoot compared to root. Thus, the applications altered the pattern of dry matter distribution favouring the shoot growth and allocated the dry matter in the shoots. In perennial (woody) plants, dry matter allocation in shoots may be a problem and reported in cherry trees under stress conditions (Aras et al., 2019), because the roots are storage organ and must be improved for the next years. However, in annual (herbaceous) plants the shoot growth is important due to dry matter distribution into fruits. In the current experiments, the pepper yield was found higher in all applications compared to control and the highest increase in the yield was provided by ZE-2 application. We consider that dry matter allocation in upper part of the plants provided higher plant yield.

Plant growth increment relies on the coordinated progression of cell division and expansion. Expansion of a tissue consists of two phases. In the first phase, cell division occurs. In the second phase, cell division has ceased and cell expansion initiates (Gonzalez et al., 2012; Aras, 2022a). In the current experiment, we evaluated pepper leaf midrib cell expansion and division. All treatments improved plant growth compared to control, parallel with that the cell expansion increased. The increase in the cell expansion may be related with the ability of nitrogen fixation of the bacteria. N influences cell expansion, growth and development (Scheible et al., 2004). Moreover, the effect of P also studied in cell division and expansion (Kavanová et al., 2006) and the authors stated that P slightly affected cell division and expansion. Therefore, the growth increment may be attributed to the N-fixing ability rather than P solubilizing. The cell division was not affected by the treatments except ZE-13+SNP. The results showed that the plant growth is a result of cell expansion rather than cell division provided by the PGPRs

and SNP. As far as we know, it is the first report on the effects of useful bacteria on plant cell physiology. SNP also affected the cell division and expansion. Many studies showed that NO promoted cell division and expansion in several plants (Han et al., 2009; Arun et al., 2017) could thus contribute to plant growth increment. NO might regulate the cell division and expansion interacting with auxin and cytokinin (Otvos et al., 2005). Midrib diameter was also assessed in the present study. Midrib consists of xylem, phloem and cortical cells has pivotal roles in water and mineral distribution (Aras et al., 2021, Aras and Endes, 2023). All treatments increased midrib diameter compared to control. The increase in the plant growth is related with the midrib size. Because, water and mineral uptake through midrib influences the plant growth and yield (Aras et al., 2021).

Principal component analysis and heatmap analysis suggest that PGPRs alone or combining with SNP improved the plant growth, cell division and expansion and yield compared to control and SNP alone application. The interpretive usefulness of PCA has been reported in many studies (Carillo et al., 2019; Kılıç, 2023).

CONCLUSION

Our results demonstrated that the PGPRs alone or combining with SNP improved the pepper plant growth and yield. SNP increased the effect of ZE-13 bacteria on cell division in leaf cells and midrib size. Furthermore, ZE-12 increased the yield combining with SNP compared to alone use. The application of PGPR with SNP could be a promising approach in plant growing.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that for this research article, they have no 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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Determining the most suitable empirical model for global solar radiation prediction in the lakes region

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Abstract

In this study, it was aimed to determine the most suitable model for predicting global solar radiation in the Lakes Region (Isparta, Burdur, Antalya). Through ATATEK-Solar software, a total of 15 models were tested, including 14 empirical models from the literature and a new artificial intelligence-supported model. Each model was analyzed with three different optimization algorithms (Nelder-Mead Simplex, Pattern Search, Simulated Annealing). In province-based evaluations, the Model 9 (RMSE: 0.1507, R^2: 0.9990) for Isparta, and the Model 14 for Burdur and Antalya (RMSE: 0.1940, R^2: 0.9992 and RMSE: 0.2218, R^2: 0.9987, respectively) provided the most successful results. In regional analysis results, while the Model 5 (RMSE: 0.2626, R^2: 0.9980) gave the lowest average error, the Model 13 (RMSE: 0.2649, R^2: 0.9979, standard deviation: 0.0122) showed the highest consistency. These models were followed by the Model 6 (RMSE: 0.2646, R^2: 0.9979, standard deviation: 0.0444). Although the Model 15 gave the best results in Burdur and Antalya, it had a high standard deviation value (0.2201) due to its low performance in Isparta. The characteristic features of the Lakes Region, including the presence of lake ecosystems, elevation differences, and the resulting microclimatic diversity, necessitate a regional approach in predicting global solar radiation. In this context, the Model 13 has been determined as the most suitable model that can be used throughout the region with its low error rate and high consistency. The obtained results can provide reliable predictions in evaluating the solar energy potential of the region and designing solar energy systems.

Keywords: Solar energy, Global solar radiation, Empirical models, Lakes Region, ATATEK-Solar

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INTRODUCTION

The continuous increase in global energy demand and efforts to combat climate change have heightened interest in renewable energy sources. Solar energy, with its low carbon emissions and high potential, is among the key sustainable energy solutions (Külcü & Ersan, 2021). Turkey benefits from a significant solar energy potential, with an annual average sunshine duration of 2,741 hours and a total global solar radiation value of 1,527.46 kWh/m² (Ministry of Energy of Turkey, 2024). Solar energy is particularly advantageous due to its abundant availability and adaptability across diverse geographical and climatic conditions.

Accurate prediction of global solar radiation is critical for the design and performance evaluation of solar energy systems. Due to atmospheric conditions, geographical features, and climatic factors, regional differences are observed in radiation predictions. Considering the installation and operational costs of solar observation devices, the regional evaluation of prediction models becomes increasingly important (Süslü & Külcü, 2024).

The Lakes Region is a geographical area located in southwestern Turkey, encompassing the provinces of Isparta, Burdur, and Antalya. The region is notable for its agricultural production and renewable energy potential. Eğirdir Lake, Burdur Lake, and other water resources influence the microclimate of the area, causing local variations in solar radiation distribution. Greenhouse farming activities in Antalya and the region's agricultural irrigation needs support the development of solar energy applications. The Lakes Region is not only vital for its

agricultural production but also for its unique geographical features that include diverse elevation levels and lake ecosystems, making it an ideal site for studying solar radiation variability. These factors underline the necessity for region-specific radiation models that account for such microclimatic differences. Antalya's extensive greenhouse farming activities require substantial energy inputs, positioning solar energy as a cost-effective and sustainable solution to meet these demands.

In the literature, various empirical models have been proposed for estimating global solar radiation. These models typically rely on parameters such as sunshine duration, temperature differences, and geographical factors. Almorox et al. (2005) examined the effects of regional adaptation of temperature-based models. Regional adaptation of solar radiation models has been shown to significantly enhance prediction accuracy, as highlighted by studies in Spain (Almorox & Hontoria, 2004) and Egypt (El-Metwally, 2005). These studies emphasize that localized optimization of model parameters improves the reliability of solar energy applications. Similarly, Külcü and Ersan (2021) conducted a study in Hatay Province, comparing the performance of different models and demonstrating that regionally optimized models provide higher accuracy.

In this study, ATATEK-Solar software was utilized to evaluate global solar radiation prediction models for the Lakes Region. This software analyzes a total of 15 models, including 14 empirical models from the literature and a new artificial intelligence-supported model (Süslü & Külcü, 2024). ATATEK-Solar facilitates regional-scale model validation and comparative performance evaluation by employing Nelder-Mead Simplex, Pattern Search, and Simulated Annealing algorithms for model optimization.

This research aims to identify a suitable prediction model for the provinces in the Lakes Region, considering the region's microclimatic characteristics and geographical structure. To achieve this goal, each model was analyzed using three different optimization algorithms, and the results were statistically evaluated. By integrating empirical models and artificial intelligence, this study not only seeks to identify the most suitable model for the Lakes Region but also aims to establish a methodological framework that can be adapted for similar regions with diverse climatic conditions. The findings obtained may contribute to the planning of solar energy applications and the development of system designs in the region.

MATERIALS AND METHODS

Study Area and Dataset

This research was conducted for the Lakes Region, located in southwestern Turkey. The selected study area comprises the provinces of Isparta, Burdur, and Antalya, which exhibit varying elevations and microclimatic characteristics (Figure 1). While Antalya is situated at an average elevation of 39 m above sea level, Isparta and Burdur are located at elevations of 1,035 m and 957 m, respectively. As shown in Figure 1, the region is characterized by lake ecosystems, which contribute to the formation of distinctive microclimatic features.

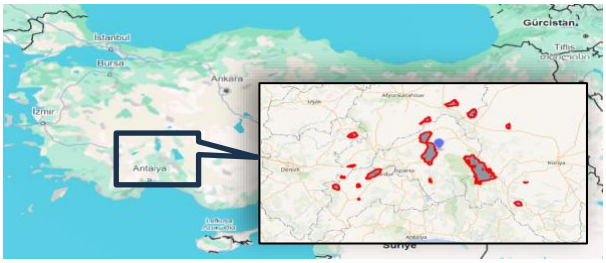

Figure 1. The location of the Lakes Region in Turkey and the distribution of its characteristic lakes.

The meteorological data used in this study were obtained from the Turkish State Meteorological Service. These long-term averaged data reflect the characteristic climatic properties of the region. The dataset includes the following parameters:

Monthly average sunshine duration (hours),
Monthly average temperature (°C),
Maximum and minimum temperature difference (°C),
Monthly total global solar radiation (MJ/m²-day),
Theoretical sunshine duration (hours),
Extraterrestrial radiation values (MJ/m²-day).

The solar radiation values were calculated as the monthly averages of daily totals derived from hourly measurements. The extraterrestrial radiation values (H_0) were computed using the following equations (Duffie & Beckman, 2006):

$$H_0 = \frac{24x3600xG_{sc}}{\pi}\left[1 + 0.033cos\left(\frac{360n}{365}\right)\right]\left[cos\varphi cos\delta sinw_s + \frac{\pi}{180}w_s sin\varphi sin\delta\right] \quad (1)$$

Where:
G_{sc}: Solar constant (1367 W/m²)
φ: Latitude angle
δ: Declination angle
w_s: Sunset hour angle
n: Day of the year (1–365)
The declination angle (δ) is calculated as:

$$\delta = 23.45sin\left[360\left(\frac{284+n}{365}\right)\right] \quad (2)$$

The sunset hour angle (w_s) is calculated as:

$$w_s = arccos[-tan(\varphi)tan(\delta)] \quad (3)$$

The climatic characteristics of the region show that Antalya has the highest annual average sunshine duration at 8.43 hours/day, with Isparta and Burdur following at 7.55 and 7.45 hours/day, respectively. The average global solar radiation values were measured as 16.90 MJ/m²-day in Burdur, 16.29 MJ/m²-day in Antalya, and 13.35 MJ/m²-day in Isparta. These differences are attributed to the topographic diversity and the microclimatic effects of the lakes in the region.

Models and Optimization Methods

Models Used

In this study, a total of 15 models were used to predict global solar radiation, including 14 empirical models commonly cited in the literature and a newly developed artificial intelligence-supported model. The model analyses were conducted using ATATEK-Solar software (Süslü & Külcü, 2024). The models examined are presented in Table 1.

Table 1. Models optimized using ATATEK-Solar.

Model No	Model	References
1	$\frac{H}{H_0} = c_1 + c_2\left(\frac{S}{S_0}\right)$	Angstrom (1924); Prescott (1940)
2	$\frac{H}{H_0} = c_1 + c_2\left(\frac{S}{S_0}\right)^{c_3}$	Elagib ve Mansell (2000)
3	$\frac{H}{H_0} = c_1^{\left(\frac{1}{S}\right)}$	El-Metwally (2005)
4	$\frac{H}{H_0} = \left[\frac{c_1\left(\frac{S}{S_0}\right)}{c_2 w_s}\right] + c_3 w_s$	Külcü (2015)
5	$\frac{H}{H_0} = c_1 + c_2\left(\frac{S}{S_0}\right) + c_3\left(\frac{S}{S_0}\right)^2 + c_4\left(\frac{S}{S_0}\right)^3$	Bahel et al. (1987)
6	$\frac{H}{H_0} = c_1 + c_2\left(\frac{S}{S_0}\right) + c_3 log\left(\frac{S}{S_0}\right)$	Ampratwum & Dorvlo (1999)
7	$\frac{H}{H_0} = c_1 + c_2 exp\left(\frac{S}{S_0}\right)$	Almorox & Hontoria (2004)
8	$\frac{H}{H_0} = c_1 + \left[c_2\left(\frac{S}{S_0}\right) + c_3\right]\varphi + c_3\left(\frac{S}{S_0}\right)$	Dogniaux & Lemoine (1983)
9	$\frac{H}{H_0} = c_1 + c_2 log\left(\frac{\frac{S}{S_0}}{w_s}\right) + c_3\left(\frac{S}{S_0}\right)$	Külcü (2019)
10	$\frac{H}{H_0} = c_1(\Delta T)^{0.5} + c_2$	Hargreaves et al. (1985)
11	$\frac{H}{H_0} = c_1 ln(\Delta T) + c_2$	Coppolino (1994)
12	$\frac{H}{H_0} = c_1[1 - exp - c_2(\Delta T)^{c_3}]$	Bristow & Campbell (1984)
13	$\frac{H}{H_0} = c_1 log\left[\left(c_2\frac{S}{S_0}\right) + (c_3\Delta T)\right] + c_4$	Külcü & Ersan (2024)
14	$\frac{H}{H_0} = c_1 log[(c_2 w_s) + (c_3\Delta T)] + c_4$	Külcü & Ersan (2024)
15	$\frac{H}{H_0} = c_1\left(\frac{S}{S_0}w_s\right)^{c_2} + c_3 log_{10}(1+\Delta T) + c_4 sin(\varphi)cos\left(\frac{2\pi n}{365}\right) + c_5$	Süslü & Külcü (2024)

Where:
H : Daily global solar radiation reaching the Earth's surface (MJ/m²-day)
H_0 : Extraterrestrial radiation (MJ/m²-day)
S : Daily sunshine duration (hours)
S_0 : Theoretical sunshine duration (hours)
ΔT : Daily maximum and minimum temperature difference (°C)
w_s : Sunset hour angle
φ : Latitude angle
n : Day of the year (1–365)
c_1, c_2, c_3, c_4, c_5 : Model coefficients

Optimization Methods

The parameters of the algorithms used for model optimization were defined as follows:

1. Nelder-Mead Simplex Algorithm: Developed by Nelder and Mead (1965), this method is widely used for solving nonlinear optimization problems.

    - Initial step size: 0.1
    - Convergence tolerance: $1x10^{-8}$
    - Maximum number of iterations: 50,000
    - Simplex size: $n + 1$ (where n is the number of model parameters)

2. Pattern Search Algorithm: Proposed by Hooke and Jeeves (1961), this algorithm is a fundamental approach among derivative-free direct search methods.

    - Initial step size: 0.2
    - Reduction factor: 0.6
    - Number of direction vectors: $2n$ (where n is the number of model parameters)
    - Maximum number of iterations: 50,000

3. Simulated Annealing Algorithm: Developed by Kirkpatrick et al. (1983), this stochastic optimization method is inspired by the annealing process in metallurgy.

    - Initial temperature: 2.0
    - Cooling rate: 0.97
    - Acceptance probability for the Metropolis criterion: $\exp(-\Delta E/T)$
    - Maximum number of iterations: 50,000

The parameter ranges for optimization were determined based on prior studies in the literature and physical constraints. In the comparison of model performance, the RMSE (Root Mean Square Error) value was prioritized; for models with equal RMSE values, the R^2 (coefficient of determination) value was evaluated.

Statistical Analysis

The evaluation of model performance was conducted using the RMSE and R^2. These parameters were calculated using the following equations:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(H_{ip} - H_{io}\right)^2} \quad (4)$$

$$R^2 = \frac{\sum_{i=1}^{N}\left(H_{ip}-H_{ipa}\right)\left(H_{io}-H_{ioa}\right)}{\sqrt{\left[\sum_{i=1}^{N}\left(H_{ip}-H_{ipa}\right)^2\right]\left[\sum_{i=1}^{N}\left(H_{io}-H_{ioa}\right)^2\right]}} \quad (5)$$

Where:
H_{ip}: Predicted value
H_{io} : Observed value
H_{ipa} : Mean of predicted values
H_{ioa} : Mean of observed values
N : Number of data points

The RMSE value indicates the magnitude of the differences between predicted and observed values. A value close to zero signifies high prediction accuracy. The R^2 value reflects the explanatory power of the model, with values closer to 1 indicating higher performance (Duffie & Beckman, 2006).

ATATEK-Solar software performs comparative statistical analyses on a monthly basis for each model. These analyses include:

- Calculation of absolute and relative differences between predicted and observed values for each month,
- Examination of seasonal performance variations of the models,
- Assessment of annual average prediction accuracy,
- Analysis of the optimized values of model coefficients and their consistency with the literature.

The selection of the ATATEK-Solar software allowed for a comprehensive evaluation of model performance using multiple optimization algorithms (Nelder-Mead, Pattern Search, and Simulated Annealing). These approaches were chosen for their proven effectiveness in solar radiation modeling studies.

Model performance comparisons were conducted using a three-step evaluation approach:

1. In the first step, models were ranked based on RMSE values.
2. In the second step, R^2 values were examined, and the explanatory powers of the models were compared.
3. In the final step, the most successful models for each province were identified and evaluated in terms of regional applicability.

This comprehensive statistical analysis approach ensures an objective assessment of both individual model performances and their applicability on a regional scale.

Determining a Common Model for the Lakes Region

A systematic evaluation process was followed to identify a common model that can represent the Lakes Region. In this process, the performance rankings of the models were first created for each province based on their RMSE and R^2 values. Subsequently, the top five models for each province were identified, and the models common to all provinces were examined for their regional consistency. For this regional consistency assessment, the average performance values of the models across the three provinces and their standard deviations were calculated.

In selecting a regional model, not only the error values of the models but also their consistency across the region were considered. To achieve this, the ranking position of each model in the provinces, its average performance across the three provinces, and the standard deviation of these performance values were evaluated together. This comprehensive evaluation approach enabled the identification of the model demonstrating the most consistent performance at the regional scale.

RESULTS AND DISCUSSION

Evaluation of Model Performance

The analyses conducted using the ATATEK-Solar software revealed the performance values of the models for each province, as presented in Table 2. The results reflect the impact of diverse climatic and geographical conditions on model performance, highlighting variations between provinces. For example, certain models exhibited higher accuracy in specific provinces due to their microclimatic characteristics.

Table 2. Performance Values of Models for Each Province

Model No	Isparta		Burdur		Antalya	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
1	0.2196	0.9980	0.3517	0.9973	0.3544	0.9966
2	0.2185	0.9980	0.2759	0.9983	0.3304	0.9970
3	2.7970	0.6711	1.8604	0.9232	1.5853	0.9314
4	0.7532	0.9761	0.6407	0.9909	0.4452	0.9946
5	0.2088	0.9982	0.2699	0.9984	0.3091	0.9974
6	0.2192	0.9980	0.2666	0.9984	0.3079	0.9974
7	0.2186	0.9980	0.4179	0.9961	0.3772	0.9961
8	0.2196	0.9980	0.3517	0.9973	0.3544	0.9966
9	0.1507	0.9990	0.3367	0.9975	0.3880	0.9959
10	0.3686	0.9943	0.4507	0.9955	0.3136	0.9973
11	0.3915	0.9936	0.4702	0.9951	0.3237	0.9971
12	0.3834	0.9938	0.4640	0.9952	0.3442	0.9968
13	0.2590	0.9972	0.2794	0.9983	0.2564	0.9982
14	0.3916	0.9936	0.4703	0.9951	0.2452	0.9984
15	0.5901	0.9854	0.1940	0.9992	0.2218	0.9987

For the analyses conducted for Isparta, the Model 9 provided the best results, with the lowest RMSE (0.1507) and the highest R^2 (0.9990). This model was followed by the Model 5 (RMSE: 0.2088, R^2: 0.9982) and the Model 2 (RMSE: 0.2185, R^2: 0.9980). These findings demonstrate the variability in model performance across regions and the importance of region-specific adaptations for accurate solar radiation predictions.

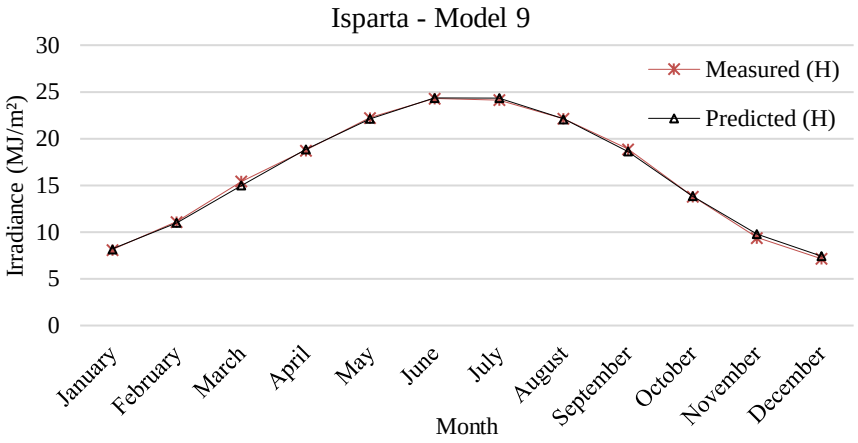

Figure 2. Best model graph for Isparta

In Figure 2, the comparison between the predicted and measured values for the Model 9 in Isparta is shown. The model's ability to accurately predict high radiation values, especially during summer months, ~~is observed.~~ underscores its adaptability to the region's microclimatic variations. This performance aligns with findings from Almorox et al. (2005), which emphasized the role of region-specific adaptations in enhancing model accuracy. Such reliable predictions are critical for optimizing agricultural planning and solar energy system placements in Isparta.

For Burdur, the Model 15 stood out with the lowest RMSE (0.1940) and the highest R^2 (0.9992). The Model 6 (RMSE: 0.2666, R^2: 0.9984) ranked second, followed by the Model 5 (RMSE: 0.2699, R^2: 0.9984). These results highlight the adaptability of Model 15 to Burdur's climatic conditions, particularly its ability to handle the transitional seasons effectively.

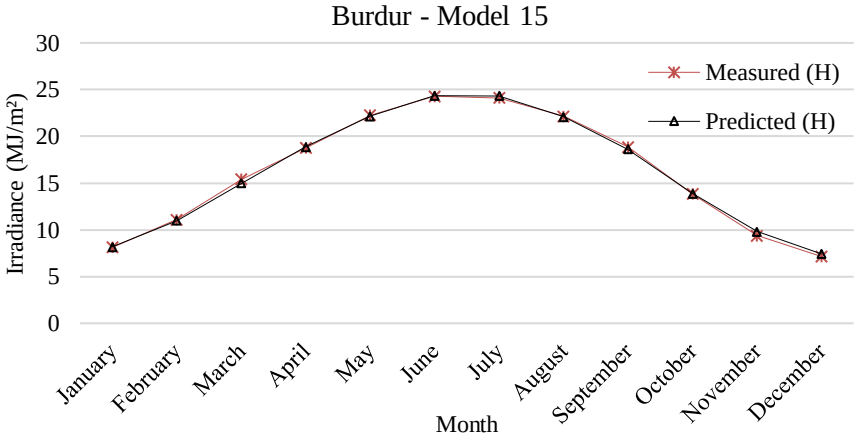

Figure 3. Best model graph for Burdur

Figure 3 presents the performance of the Model 15 for Burdur. As shown in the graph, the model closely follows the measured values throughout the year, achieving accurate predictions, particularly during transitional seasons. This performance is critical for solar energy applications in Burdur, where transitional seasons often affect energy demand and supply dynamics.

For Antalya, the Model 15 also provided the most successful results (RMSE: 0.2218, R^2: 0.9987). The Model 14 (RMSE: 0.2452, R^2: 0.9984) and the Model 13 (RMSE: 0.2564, R^2: 0.9982) showed the second and third-best performances, respectively. The high accuracy of Model 15 highlights its robustness in capturing Antalya's complex climatic conditions, particularly during the high-radiation summer months.

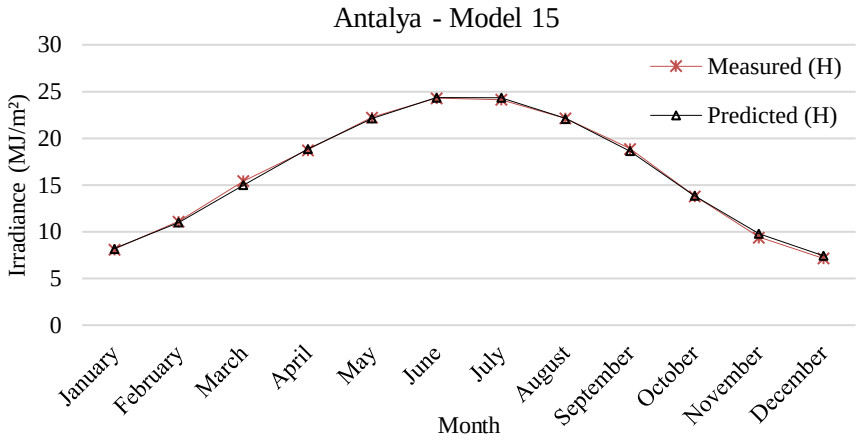

Figure 4. Best model graph for Antalya

Figure 4 illustrates the predictive performance of the Model 15 for Antalya. The model successfully captures Antalya's characteristic solar radiation profile and accurately reflects seasonal variations. This level of precision is crucial for planning solar energy projects, especially in Antalya, where the agricultural sector heavily relies on efficient energy usage during peak seasons.

Regional Model Performance Analysis

To evaluate the performance consistency of the models across the Lakes Region, the average performance values of each model for the three provinces and their standard deviations were calculated (Table 3).

Table 3. Regional performance metrics of models

Model No	Average RMSE	Standard Deviation	Average R^2
1	0.3086	0.0773	0.9973
2	0.2749	0.0559	0.9978
3	2.0809	0.6334	0.8419
4	0.6130	0.1555	0.9872
5	0.2626	0.0511	0.9980
6	0.2646	0.0444	0.9979
7	0.3379	0.1060	0.9967
8	0.3086	0.0773	0.9973
9	0.2918	0.1219	0.9975
10	0.3776	0.0693	0.9957
11	0.3951	0.0735	0.9953
12	0.3972	0.0610	0.9953
13	0.2649	0.0122	0.9979
14	0.3690	0.1156	0.9957
15	0.3353	0.2201	0.9944

These metrics provide valuable insights into the models' regional performance and consistency, which are critical for identifying models suitable for diverse microclimatic conditions in the Lakes Region. The relationship between the regional performance and consistency of the models is visualized in Figure 5. In the graph, the horizontal axis represents the average RMSE values, while the vertical axis shows the standard deviation values. Models located near the bottom-left corner of the graph demonstrate both low error and high consistency.

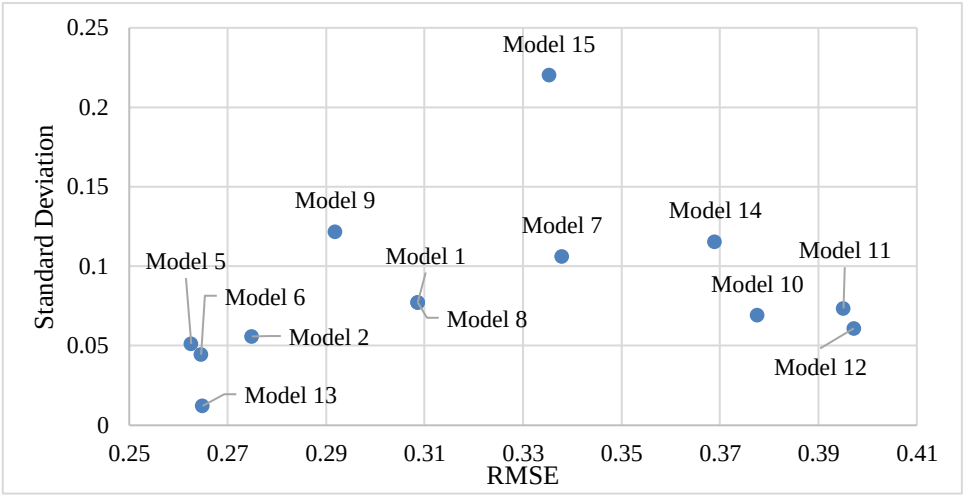

Figure 5. Distribution of Average RMSE and Standard Deviation Values of the Models

According to the results of the regional analysis, the Model 13 demonstrated the most consistent performance across the region, with the lowest standard deviation (0.0122). The stability of Model 13 aligns with findings from previous studies, where regionally adapted models demonstrated high consistency across diverse climatic conditions (Almorox et al., 2005). This was followed by the Model 6 (standard deviation: 0.0444) and the Model 5 (standard deviation: 0.0511), both of which also produced stable results regionally. This highlights the robustness of these models in handling the diverse microclimatic conditions present in the Lakes Region. This is clearly shown in Figure 5, where these models are clustered in the lower-left corner of the graph.

Although the Model 15 performed best in Burdur and Antalya, its relatively poor performance in Isparta resulted in a high standard deviation (0.2201). Similarly, while the Model 9 achieved the best results in Isparta, its performance declined in the other provinces. These results suggest that while certain models excel in specific provinces, they may lack adaptability across the entire region, emphasizing the importance of selecting models with both accuracy and consistency.

When examining the average RMSE values of the models, the Model 5 had the lowest value (0.2626), followed by the Model 13 (0.2649) and Model 6 (0.2646) models. These three models also showed high explanatory power, with average R^2 values exceeding 0.9978. Such performance indicates their potential application in regional energy planning and agricultural optimization, where reliable solar radiation predictions are critical.

CONCLUSION

In this study, 15 different models were tested to determine the most suitable model for predicting global solar radiation in the Lakes Region. The performance of the models was analyzed using the ATATEK-Solar software with three different optimization algorithms, and the results were evaluated at both the provincial and regional levels.

In the provincial evaluations, the Model 9 provided the best results for Isparta, with the lowest RMSE (0.1507) and the highest R^2 (0.9990). In contrast, the Model 15 performed best for Burdur and Antalya, with RMSE and R^2 values of 0.1940 and 0.9992, and 0.2218 and 0.9987, respectively. However, performance differences in other provinces limited the regional applicability of these models.

According to the results of the regional analysis, the Model 5 and the Model 13 were identified as the two most successful models. The Model 5 achieved the lowest average error (RMSE: 0.2626, R^2: 0.9980), while the Model 13 demonstrated similar performance with an RMSE of 0.2649 and R^2 of 0.9979. These two models were followed by the Model 6, with an RMSE of 0.2646 and R^2 of 0.9979.

When examining regional consistency, the Model 13 produced the most stable results, with the lowest standard deviation (0.0122). This value indicates that the model can provide consistent predictions despite varying climatic and geographical conditions in the region. The Model 6 (standard deviation: 0.0444) and the Model 5 (standard deviation: 0.0511) also exhibited stable performance across the region.

Although the Model 15 produced the best results in some provinces, its high standard deviation (0.2201) indicated weaker regional consistency. Similarly, while the Model 9 achieved high performance at the provincial level, it did not generate consistent results across the region.

Considering the characteristic features of the Lakes Region, including the presence of Eğirdir Lake, Burdur Lake, and other water bodies, as well as the elevation differences and the resulting microclimatic diversity, a regional approach is necessary for global solar radiation prediction. In this context, the Model 13 stands out as the most suitable model for regional use, with its low error rate and high consistency. The model provides acceptable error levels for all three provinces and produces reliable predictions under varying climatic conditions in the region.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

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.

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Improving seed germination and bulb induction of *Allium tuncelianum* kolmann under aseptic conditions

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Abstract

Allium tuncelianum (Kollman) N. Ozhatay, B. Mathew & Siraneci] or Tunceli garlic is endemic to the Eastern Turkish Provinces of Tunceli, Sivas Erzincan and [Munzur mountains]. They are edible and bear attractive deep lilac colored flowers with fertile black deep dormant seeds. Tunceli garlic seeds were collected from field-grown plants and aimed to break seed dormancy to optimize conditions for induction of bulblets, along with their growth, development, and increased bulb diameter. Therefore, these were cultured on MS medium amended with different strengths of KNO_3. They were germinated on MS medium with or without 20 g L^{-1} sucrose followed by their culture on 1, 2, 4 and 6 × KNO_3 (found in MS medium) to increase bulb diameter. Improved seed germination was noted on MS medium with and without sucrose but with variation compared to the previous reports. The bulb formation rate on each of the germinated seeds was not parallel. The results showed 34 and 28.5% bulb induction noted on germinated seeds after 150 and 158 days on MS medium containing 20 g L^{-1} sucrose and no sucrose in the same sequence. The results emphatically noted the role of cold stratification on agar-solidified MS medium supplemented with sucrose to improve seed germination. The best increase in bulb diameter was noted on MS medium containing 1 × KNO_3 (found in MS medium) after 178 days with bulblet diameter and weight of 0.54 cm and 0.048 g, respectively. Consequently, the bulbs induced on sucrose-containing MS medium could be transferred to pots earlier. Increased (>1 × KNO_3 found in MS medium) negatively affected on the growth and development of Tunceli garlic bulbs. The strategy of seed germination and bulblet induction reported in this study could be positively used to conserve and protect this endemic plant species.

Keywords: Tunceli garlic, Seed, dormancy, Bulblets, Bulb growth

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INTRODUCTION

Tunceli garlic [*Allium tuncelianum* (Kollman) N. Ozhatay, B. Mathew & Siraneci] with white-to-purple or lilac colored inflorescences on single-gloved, cream-white bulbs are endemic to Eastern Turkish Province of Tunceli and the surrounding Munzur mountains (Baktir, 2005; Yanmaz and Ermis, 2005). Tunceli garlic bears fertile black seeds that can easily be used for propagation (Arslan et al., 2010); however, they undergo deep seed dormancy soon after maturity. The bulbs are generally produced asexually by bulbil propagules attached to the main body of mother bulbs. Propagation by seed can produce mature plants after 2 – 3 years under ideal conditions of growth and development.

Like all allium species, Tunceli garlic also contains a biologically active organic sulfur compound, allicin (thio2-propene-1-sulphinic acid S-allyl ester). Allicin has been reported to have anti-coagulant, anti-hypertensive, antimicrobial, anti-biotic, anti-parasitic, anti-mycotic, anti-viral, anti-tumor, anti-oxidant, and anti-aging activities (Jacob, 2006; Ozkan et al., 2013). Allicin is also known to detoxify heavy metals, be hypolipidaemic (i.e., lipid-

lowering), anti-carcinogenic, and antimutagenic characteristics (Ozkan et al., 2013; Munchberg and Anwar, 2007; Iciek et al., 2009; Kizil et al., 2014).

Dormancy can be broken with the cold treatment given to garlic before planting it. Garlic growth is slow when it begins its vegetative activity, because the plant needs a cold moist climate, with nocturnal temperatures between 8 and 20 °C and diurnal temperatures of 13 and 24 °C to grow vigorously (Rahman et al., 2003).

The low temperatures that plants receive in the period before bulb induction determine the beginning of vegetative growth which obviously affects yields. With temperature of 0 °C, the plants are no longer able to take nutrients from the soil and remain in dormancy until temperatures rise.

The bulb formation stage in garlic begins when the average soil temperature is around 18 to 20 °C. Better induction and growth of bulbs are observed on long days with high temperatures compared to short days with low temperatures. There is no report on seed germination or seed dormancy break of Tunceli garlic. This situation complicates the cultivation of the plant by seed.

Therefore, the study aimed to break the seed dormancy of Tunceli garlic, induce new bulblet formation and increase bulb diameter by culturing germinated seeds on different strengths of KNO_3 in MS medium.

MATERIALS AND METHODS

Tunceli garlic seeds were obtained from the Experimental Gardens of the Department of Field Crops, Dicle University, Diyarbakir, Turkey. They were washed in slow - flowing tap water to remove all dirt and soil. The seeds were tested for seed viability before seed germination test using 2,3,5-Triphenyl-tetrazolium chloride. The 1000 seed weight of Tunceli garlic was determined as 2.13 g. Then, the seeds were surface sterilized using 100 % (v/v) bleach (containing 5 % (v/v) NaOCl) for 20 min. The seed viability of Tunceli garlic was tested using 1 mg ml^{-1} 2,3,5 - triphenyl tetrazolium chloride, by penetration and immersion of living tissues of longitudinally cut seed embryos in the dark at 24 °C. The seed viability was noted after 24 hours by observing the formation of insoluble reddish formazan.

Thereafter, the seeds were rinsed 3 × 3 min in distilled sterilized water. The seeds were cultured on MS medium (Murashige and Skoog, 1962) supplemented with and without 2.0 % (w/w) sucrose solidified with 0.62% (w/w) agar (Duchefa – Haarlem, The Netherlands) at 4 ±1°C (Table 1).

The germinated seeds were cultured on MS medium containing 1, 2, 4 and 6 × KNO_3 (found in MS medium) supplemented with 30 g L^{-1} sucrose to increase bulb diameter. After 8 weeks in culture, the rooted bulblets were transferred to pots containing peat moss. The bulblets in pots were initially grown under controlled environmental conditions for 10 d in a growth chamber to acclimatize them followed by their transfer to the greenhouse.

The pH of all cultures was adjusted to 5.7±0.1 using 0.1 M NaOH or 0.1 M HC1 before autoclaving. All media were autoclaved at 104 kPa pressure and 121 °C for 20 min. All cultures were incubated in the refrigerator at 4 ± 1 °C in the dark during seed germination in a growth chamber (Fitotron SGC 120-United Kingdom) at 24 ± 1ºC with a 16 h light photoperiod during bulblet induction.

RESULTS AND DISCUSSIONS

Seed viability of Tunceli garlic was made using 2,3,5 triphenyl tetrazolium chloride, a test developed for rapid seed viability testing. A penetration of 2,3,5 triphenyl tetrazolium chloride in living tissues of longitudinally cut-through embryos and cotyledons resulted in the reduction of hydrogen ions released by enzymes involved with the respiration process. This resulted in the formation of an insoluble reddish formazan compound after 24 hours of incubation at 24 °C in agreement with Youngblood (2008), Sosnoskie and Cardina (2009), and Miller and Peters (2010).

The result showed that 60.0% of seeds (240/ 400 seeds) were viable; as their embryonic axes and the cotyledon tissue were fully stained red by masking their dirty white color.

Surface sterilized seeds germinated on MS medium with and without 20 g L^{-1} sucrose at 4 ±1°C in dark showed average seed germination of 56.5% and 51.5% (Table 1; Figure 1a) respectively. Similarly, seed germination on MS medium with and without 20 g L^{-1}sucrose started after 81 and 85 days of culture, which continued till 110 and 117 days, respectively (Table 1 and 2).

The bulb formation rate on each of the germinated seed was not parallel to the seed germination rate. A total of 34% seeds (with 138 seeds that converted to bulbs) (Figure 1b) and 28.5% (with 94 seeds that converted to bulbs) on MS medium with and without 20 g L^{-1} sucrose respectively (Table 2). Previous studies suggest that storage at low temperatures has sharp and significant effects on breaking seed dormancy of garlic (Cantwell et al., 2003; Vazquez et al., 2006). Similarly, Arguello et al. (2001) also suggested that garlic seeds treated at a 4°C and Gibberellic Acid (GA_3) were helpful in seed dormancy break. It is important to know that storage at 4°C results in the hydrolysis of seed starch resulting in carbohydrate mobility in *Allium sp*. (Fulton et al., 2001). This results in the conversion and transport of macro carbohydrate molecules into sucrose, glucose, and fructose, with accumulation of these macromolecules ends up into energy that is utilized during metabolism of cells and energy required for the growth of plants (Hapkins, 1999; Langens et al., 2003).

Table 1. Observation of *A. tuncelianum* seeds under *in vitro* conditions supplemented with different sucrose amounts

Details	MS medium with sucrose	MS medium without sucrose
Seed germination percentage (%)	56.5	51.5
Number of germinated seeds	226	206
Start of *in vitro* seed germination (days)	81	85
End of *in vitro* germination (days)	110	117
Bulb formation (days)	150	158
Bulb formation (%)	34	28.5
Number of bulbs transferred to pots	136	94.0
Bulb transfer to pots (days)	178	196

Table 2. Effect of KNO_3 concentration on bulb growth of *Allium tuncelianum* bulblets

Strength (×) of KNO_3 in MS medium g L^{-1}	Average bulb diameter (mm)*	Average bulb weight (g)	Root induction
1 ×	0.54 a	0.048	+
2 ×	0.47 b	0.036	+
4 ×	0.41c	0.038	+
6 ×	0.35d	0.028	-

* Each figure is mean of 10 × 3 bulbs

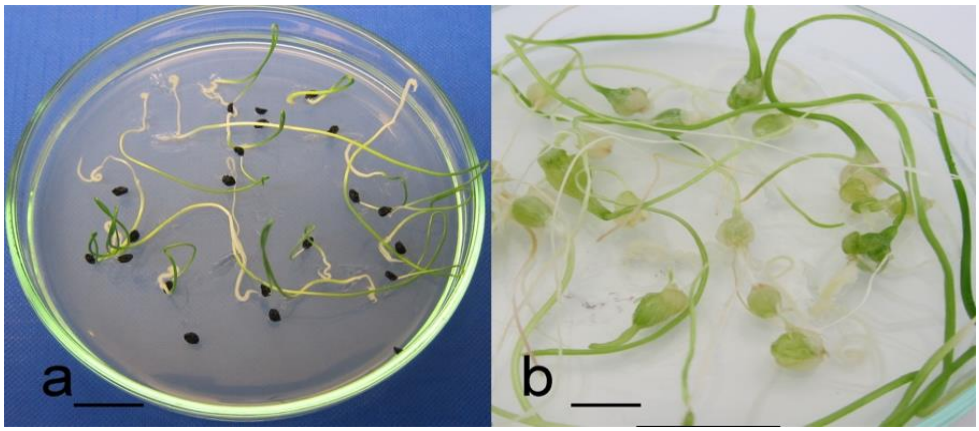

Figure 1. Seed germination of Tunceli garlic under *In vitro* conditions (a) surface sterilized seeds germinated on MS medium (b) bulb formation on 20 g L^{-1} sucrose at 4 ±1°C in dark, bar of Fig 1a=1.5 cm , Bar of Figure 1b=0.75 cm

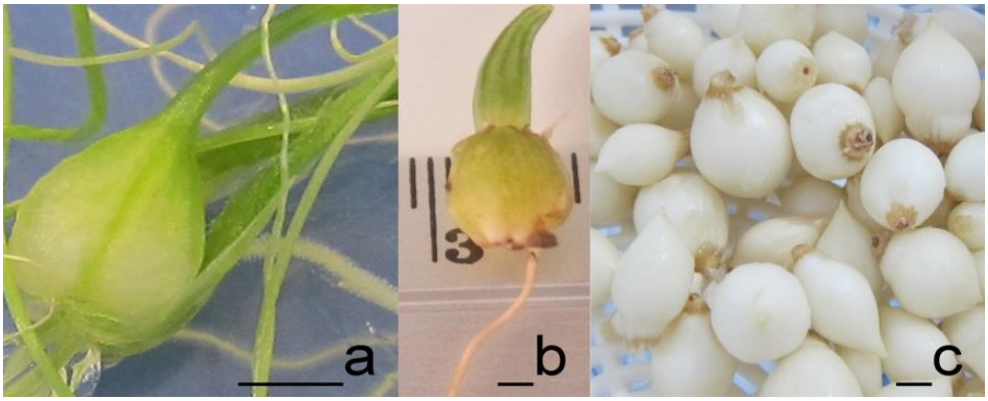

Figure 2. Tunceli garlic bulblets under *In vitro* conditions (a) increase in bulb diameter (b) the rooted bulbs induced on MS medium with sucrose after 178 days on culture before transfer to pots and nature bulbs of *A. tuncalianum*; Bar a, b=1 cm, c= 0.4 cm, d=0.7 cm

The bulbs induced on MS medium with sucrose took 178 days in their transfer from in vitro conditions to pots (Figure 2a, b). The bulbs that were not induced on 20 g L^{-1}sucrose took a longer time and could be transferred to pots in 196 days only. This showed that MS medium containing sucrose had a significantly positive effect on seed germination, bulb induction and vegetative growth.

Bulbs induced on MS medium were transferred to 1, 2, 4 × and 6 × KNO_3 in MS medium (Table 2) supplemented with 30 g L^{-1} sucrose. A positive increase in bulb diameter and weight in the range of 0.35 to 0.54 cm and 0.28 to 0.48 g was noted on different strengths of KNO3-supplemented media. Each increase in the strength of KNO_3 was inhibitory and affected bulb diameter negatively. Maximum increase in bulb diameter was only noted on supplementing 1× KNO_3 in MS medium. The results showed both reductions in bulb diameter and weight on increased 6 × KNO_3 (found in MS medium) and consequently, no rooting was noted on bulbs cultured on MS medium supplemented with 6 × KNO_3 (found in MS medium). Bulb diameter in the range of 0.41-0.54 cm and bulb weight in range of 0.038 - 0.48 cm was favorable for rooting (Fig 1d). Rooting was noted on all bulbs cultured on MS medium supplemented with 1, 2 × and 4 × KNO_3 (found in MS medium). Volk (2009) suggests that garlic (*A. sativum*) cultures grown under diverse conditions are affected by soil potassium levels. Potassium levels are positively correlated with bulb circumference and fresh weight. Similarly, Arguello et al. (2001) suggested that garlic micro-bulblets physiologically mature and are able to sprout after 90 days of storage. Cloves exposed to 5°C for 15–30 days before sowing accelerated the maturity of bulbs compared with cloves cultured at room temperature or 20°C Rahim and Fordham, 2001). Dufoo-Hurtado et al. (2013) emphasize that pre planting cold storage at 5°C for 5 weeks resulted in significant improvement in maturity and purple color enhancement of garlic bulbs.

Each of the induced bulbs was cultured on 0, 30, 60 and 90 g L^{-1} sucrose to increase bulb diameter. No statistical differences were noted among the number of shoots per bulb, and shoot length due to changing concentrations of sucrose. Maximum bulb diameter was noted on 30 g L^{-1} sucrose. Whereas, maximum bulb weight was noted on 90 g L^{-1} sucrose. All concentrations of sucrose induced variable number of roots. Kumar et al (2005) noted that bulbscales of Star Gazer hybrid induced bulblets on MS medium with growth retardants, and several sucrose concentrations, and exposure to light or darkness. Alar, Cycocel, and Paclobutrazol increased number of bulblets but decreased with increased sucrose additives, and none of them produced leaves in continuous dark.

Subsequent culture of these bulblets (the bulblets cultured on 30 g L^{-1} sucrose) on MS medium using 30 g L^{-1} sucrose for 20 – 22 weeks showed further improved the morphological parameters. Each bulb developed average number of 0.7 shoots with a shoot length of 2.30 cm, bulb diameter of 0.63 cm, bulb weight of 0.21 g, and rooting percentage of 83.3%.

The results are in partial agreement with Pooler and Simon (1994), who reported that garlic seeds, stored at 3ºC for 1–2 months, had a germination rate of 10% only. However, the seeds, harvested in spring, showed 80% germination when they were stratified at 0–3 ºC for 2 weeks in cool and humid conditions. They transferred seeds to 22°C temperature under 16 h long light conditions after seed germination. The results show improvement over previous reports by Etoh and Simon (2002) and are in partial agreement with them. They reported 20% seed germination by storing the seeds at 3ºC for 3–6 months followed by transfer to 5ºC. They noted treatment with of phytohormones was not suitable for seed germination. They confirmed that garlic seeds need stratification at low temperatures. Yanmaz and Ermis (2005) tried to break seed dormancy in Tunceli garlic using variants of GA_3 (0.5, 1.0 and 2 mg L^{-1}) for 30 to 90 d at 0 - 5°C and achieved 20% seed germination only. They emphasize that stratification under moist conditions had an edge over GA_3 treatments in seed germination.

CONCLUSIONS

- ✓ The seed germination rate was higher under both conditions mentioned in the experiment compared to the previous reports.
- ✓ Cold stratification on agar-solidified MS medium was needed to improve seed germination.
- ✓ Supplementation of sucrose in the germination medium reduced the seed germination to 81-110 days after culture.
- ✓ No supplementation of sucrose in the germination medium delayed the seed germination to 85-117 days after culture. The seed germination rate was parallel to the seed viability rate.
- ✓ Bulbs induced on sucrose-containing MS medium were transferred after 178 d of culture to pots.
- ✓ Bulbs induced on non-sucrose-containing MS medium were transferred only after 196 d of culture to pots.
- ✓ The best bulblet diameter and weight induction was noted on MS medium containing 1 × KNO_3 (in MS medium) + 30 g L^{-1} sucrose.
- ✓ The study describes a simple procedure for the conservation of this significantly important plant species.

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.

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Determination the effective dose of mutation in pepper (*Capsicum annum* L.)

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Abstract

This study was carried out to determine the "Effective Dose of Mutation" (EMD_{50}) for mutation breeding study on Çermik, Kandil and Üçburun pepper varieties grown intensively in Diyarbakır. For this purpose, pepper seeds were exposed to chemical mutagen source Ethyl Methane Sulfonate (EMS) at doses of 0%, 01%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% and 1% and to irradiation with physical mutagen source Co^{60} at doses of 0, 50, 100, 200, 300, 400, 500 and 600 Gy. In irradiated and chemically exposed seeds, on the 45th day following seed sowing date, germination percentage (%) for each dose was calculated and seedlings' lengths were measured to investigate the effects of different doses and EMD_{50} value was calculated. According to the data obtained as a result of the study; it was found that the germination rate in pepper seeds of all three varieties-genotypes gradually decreased with the increases of doses in application of EMS on pepper seeds. While the decreases were especially evident in the seeds of Kandil and Üçburun varieties subjected to 0.3% EMS dose and 9 hours of application; in Çermik genotype the germination rate started to decrease with 0.5% EMS dose due to local population. It was also found that germinations decreased in all three varieties depending the application of 300 Gy CO^{60}. In 6-hour EMS application, EMD_{50} values were respectively determined as 0.67%, 0.97% and 1.08%. for Kandil, Üçburun and Çermik. And for 9-hour EMS application, EMD_{50} values were determined as; 0.52%, 0.77%, 0.89%. Kandil, Üçburun and Çermik varieties. According to the results obtained in the study, the usage of both physical and chemical mutagens in pepper varieties; increasing of doses and durations of mutagens, decreased germination rates and also caused to decrease the plant heights. As a result, it seems possible to obtain variations in peppers with the usage of physical and chemical mutagens to get new varieties in plant breeding.

Keywords: Pepper, Effective Mutation Dose, Gamma, Ethyl Methane Sulfonate, Germination

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INTRODUCTION

Pepper (*Capsicum* sp.), is an important species of the *Solanaceae* family that has an important position among other vegetables in terms of production area and production amount in the world and in Türkiye. According to 2022 data from the Food and Agriculture Organization of the United Nations (FAO), 36,972,410 tons of pepper produced in the area of 2,020,816 hectares in the world. According to these data, an average yield of pepper is 1,83 kg/ m2 (FAO, 2022). The countries those have the most pepper production in the world are respectively; China is first with 16,837,405 tons, Mexico is the second with 3,113,244 tons, Indonesia is third with 3,020,262 tons, Türkiye is fourth with 3,018,775 tons and Spain is fifth with 1,533,280 tons. According to these results, China alone produces half of the world's pepper production.

When we look at the pepper varieties produced in Türkiye, capia was the most grown and produced pepper in 2023 (Table 1). According to table 1, it was determined that the average yield of capia pepper is 4,27 tons/decare the long pepper is 3,73 tons/decare, bell pepper is 3,46 tons/decare and Charleston pepper is 6,78 tons/decare in Türkiye (TUİK, 2023).

Table 1. The pepper varieties grown in Türkiye

Varieties	Growing Area (Decare)	Production (Tonnes)
Capia (Paste)	375,147	1,602,457
Long	251,672	939,178
Green Bell	114,197	395,441
Charleston	21,218	143,934

Anonymous: TUIK, 2023

While to show the ability of obtaining high yields from a unit area as mathematically; "Yield = Plant's Genetic Potential + Cultivation Technique Package + Environmental Conditions" can be formulated (Kurt, 2015). It is an expected result that the high yield obtained from a unit area due to the seed containing high yield that is the source of plant production. Therefore, the seed will be used it is expected to strong in terms of desired features (quality, market value, high yield, resistance to diseases and pests, etc.).

When the seeds used in commercial production in the world and in Türkiye were investigated, it was seen that the majority of the seeds used were F_1 hybrid seeds (Tepe et al., 2003; Kantoğlu, 2014a). F_1 hybrid seeds are obtained as a result of crossbreeding between parents with superior characters that are not related in terms of genetic structure. Hybrid seeds obtained by hybridization (Heterosis) can have very different and high-quality characteristics from their parents (mother and father) (Hayward et al., 1993; Kantoğlu, 2014a). For example; yield, quality, resistance to viral and fungal diseases, resistance to pests, adaptability to environmental conditions, etc. While the most of companies selling F_1 seeds in Türkiye in the past were foreign companies, the number of domestic companies has been increasing day by day with the increase in the number of qualified personnel working in plant breeding. With the presence of local genotypes and improving new varieties seeds in many vegetable species in Türkiye, the demand for foreign companies is gradually decreasing, and thanks to local companies in the country, the production of many types of hybrid seeds improved in many different varieties.

In the world and in Türkiye breeding studies began with classical breeding and tissue culture methods (Abak, 1983) and today continue with more advanced technologies. In order to eliminate the negative effects that still occur in varieties obtained with different methods (classical breeding, anther culture, biotechnological methods, etc.), researchers have tried to create new genetic variations using an alternative method; different physical and chemical mutagens (agents that change the genetic structure of an organism) (Kökpınar et al., 2021).

Physical and chemical agents that artificially initiate hereditary changes to create variation in plants increase genetic diversity in plants and help to create new mutant lines with improved characteristics (Krupa-Małkiewicz et al., 2017). While physical mutagens cause more chromosome changes and larger DNA deletions, chemical mutagens usually cause point mutations (Okagaki et al., 1991; Anonymous, 2024a).

More than 70% of the mutant varieties obtained and sold in the market to date have been obtained by using physical mutants. The most commonly used physical mutation source has been gamma rays. Mutation breeding was first performed in pepper in 1940 using X-rays (Daskalov, 1986). In later years, many researchers obtained genetic variations using different physical and chemical mutagens (Micke and Donini, 1993; Lambat et al., 2012; Sikder et al., 2013; Sikder et al., 2015; Cheng et al., 2019; Dhamayanthi and Reddy, 2000; Xu et al., 2020; Soyam, 2021; Tanaka et al., 2021; Maurya and Bahadur, 2022; Dongfu et al., 2022; Singh et al., 2022). Mutation breeding, which is a method frequently used by plant breeders, is known to be an important method in terms of its lower cost, providing a large number of genetic variations, and especially in the development of characters that show simple heredity in self-pollinated plants (Sagel et al., 2002; Gerami et al., 2017). The frequency of mutation that will occur with physical or chemical mutagen application can be 103 times higher than the mutation frequency that will occur naturally (Kantoğlu, 2014b). However, in mutation breeding, the emergence of new lines and their detection can take a long time and with great difficulty. Since mutation breeding also occurs at low frequencies, if a large number of plant materials (thousands of plants) are started, the formation of a new and desired character is possible (Anonymous, 2024b).

As a result of studies using different physical and chemical mutation methods, many species and varieties with the desired characters (dwarfism, high yield and quality, resistance to diseases and pests, etc.) have been developed by many public and private companies in the world and in Türkiye (Tantray et al., 2017; Kazaz and Kholmurotov, 2022). Mutation breeding, due to its easy applicability, non-hazardous and environmentally friendly, fast and proven breeding method, has been commercially used in more than 210 plant species in more than 70 countries and more than 3.400 mutant plant varieties. The product with the most mutant variety production in the world is rice (873 units), followed by barley (307 units), chrysanthemum (285 units), wheat (265 units) and soybean (182 units) is the fifth plants. The Asian region ranked first with 2.087 mutant varieties produced, followed by Europe with 960, North America with 211, Africa with 82, and Latin America with 53 mutant varieties. The most developed country in the world with mutant varieties was China. China, which had 817 mutant varieties, was followed by Japan with 500 varieties and India with 345 varieties (Anonymous, 2024c). The number of mutant pepper varieties produced and registered in the world was 16 (Table 2). In Türkiye, mutant varieties were registered in 19 different plant species (Anonymous, 2024d). When table 2 was examined, it was seen that there was no

registered variety in our country, but it was seen in the literature that many studies had been carried out since ancient times under the leadership of Türkiye Atomic Energy (Tepe et al., 2003; Taner et al., 2004; Beşirli et al., 2007; Kantoğlu et al., 2010; Kantoğlu et al., 2014a-b, Sarıçam et al., 2017; Kantoğlu et al., 2018; Büyükdinç et al., 2019; Aziz et al., 2021).

Table 2. Mutant pepper varieties produced in the world.

Variety Name	Latin Name	Common Name	Country Name	Registration
Albena	*Capsicum annuum* L.	Green pepper	Bulgaria	1976
F_1 Orange Beauty	*Capsicum annuum* L.	Vegetable Pepper	Russian Federation	2011
Friar KS80	*Capsicum annuum* L.	Green pepper	Italy	1985
Gornooriahovska door	*Capsicum annuum* L.	Pepper	Bulgaria	1997
Horgoska slatka-X-3	*Capsicum annuum* L.	Pepper	Serbia	1974
Krichimsky ran	*Capsicum annuum* L.	Green pepper	Bulgaria	1972
Ljulin	*Capsicum annuum* L.	Green pepper	Bulgaria	1982
Longjiao 9	*Capsicum annuum* L.	Pepper	China	2005
MDU.1	*Capsicum annuum* L.	Chilli	India	1976
Nush-51	*Capsicum annuum* L.	Sweet pepper	Russian Federation	1991
Orangeva Door to door	*Capsicum annuum* L.	Sweet pepper	Bulgaria	1991
Rice	*Capsicum annuum* L.	Sweet pepper	Bulgaria	1991
Yujiao 1	*Capsicum annuum* L.	Pepper	China	2002
Yujiao 2	*Capsicum annuum* L.	Pepper	China	2006
Yujiao 3	*Capsicum annuum* L.	Pepper	China	2007
Yujiao 4	*Capsicum annuum* L.	Pepper	China	2007

Source: Anonymous, 2024c.

The plant species produced in Türkiye and registered by the IAEA are shown in Table 3. It has been seen that most of the studies were on fruit species in Türkiye.

Table 3. Plant species recorded by the IAEA in Türkiye.

Variety Name	Latin Name	Common Name	Country Name	Registration
Bademler Beyazı	*Chrysanthemum x morifolium Ramat*	Bademler Beyazı	Türkiye	2022
Ege Meltemi	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Kaan	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Ozan	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Nahita	*Solanum tuberosum* L.	Potato	Türkiye	2016
Önder	*Hordeum vulgare* L.	Barley	Türkiye	2016
Aldamla	*Prunus avium* L.	Cherry	Türkiye	2014
Burak	*Prunus avium* L.	Sweet cherry	Türkiye	2014
Birkan	*Sesamum indicum* L.	Sesame	Türkiye	2011
ALATA	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Gülşen	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Uzun	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Eylül	*Citrus limon* L. Burm.	Lemon	Türkiye	2009
TAEK-SAGEL	*Cicer arietinum* L.	Chickpea	Türkiye	2006
TAEK-PESKIRCIOGLU	*Nicotiana tabacum* L.	Tobacco	Türkiye	1999
TAEK-TUTLUER	*Nicotiana tabacum* L.	Tobacco	Türkiye	1999
Akdeniz M-Q-54	*Hordeum vulgare* L.	Barley	Türkiye	1998
TAEK A3	*Glycine max* L.	Soybean	Türkiye	1994
TAEK C10	*Glycine max* L.	Soybean	Türkiye	1994

Anonymous, 2024c

It is seen in the examination of academic studies that many studies have been conducted on the use of mutation breeding in peppers in the world. Although the number of pepper varieties registered and recorded by the IAEA seems to be low, it is known that there are still many studies in the breeding process around the world. In order to determine the targeted characteristics in the studies, it is very important to create the variation to obtain with the correct dose of mutagen (physical or chemical) applications. It should not be forgotten that the LD^{50} dose and dose limits can be vary for each genotype to genotype (Spencer-Lopes et al., 2018; Çelik et al., 2021). In order to develop an effective variation, determining the LD^{50} mutagen dose specific to each variety or genotype before starting the mutation breeding project increases the success of the study (Kantoğlu, 2022). Some of the academic studies on mutation breeding in pepper are as follows.

Dongfu et al. (2022), in pepper (*Capsicum annuum*) used EMS chemical mutagens to increase genetic variations. At the end of the study, it was reported that different structural variations were obtained at different doses. Soyam (2021) used different doses of EMS (0.2, 0.3 and 0.4%) to increase chlorophyll in pepper and ascorbic acid content, it was stated that as the EMS doses increased, the ascorbic acid content decreased and the amount of chlorophyll-a increased. Tanaka et al. (2021) applied EMS to obtain seedlessness in pepper and stated that they obtained seedless lines at the end of the study. Cheng et al. (2019) applied ethyl methane sulfonate to determine the physico-biochemical characterization of leaf color in pepper. At the end of the study, it was stated that leaf colors deteriorated according to the doses. Dhamayanthi and Reddy (2000) applied gamma rays (15, 25, 35 kR) and EMS (They investigated the effects of 0.8% and 1%) applications on the plant. EMS has been reported to be more effective in inducing meiotic irregularities than gamma rays. Lambat et al. (2012) reported that gamma rays (10 KR, 20 KR and 30 KR) and ethyl methane sulfonate (0.1%, 0.2% and in their studies investigating the cytological and morphological effects of 0.3%) on pepper, they reported that it induced various nuclear and chromosomal abnormalities. Sikder et al. (2015) reported that they used gamma rays and EMS mutagen on tomatoes, and that as a result the increase in both mutagens, seed germination, seedling height and number of pollen decreased. As a result of there was a decrease in productivity too. Sood et al. (2016) applied gamma ray (0.5 kR, 1.0 kR, 3.0 kR, 5.0 kR, 8.0 kR, 11 kR, 13 kR, 16 kR, 19 kR and 22 kR) and EMS (0.1%, 0.25%, 0.50%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% and 3.0%) doses, there were decreases in germination, root length, shoot length and seed emergence rate. It has been stated in the studies that UV rays are also used in mutation breeding (Anonymous, 2024e) Rodríguez-Calzada et al. (2019) UV -B radiation in hot pepper (*Capsicum annuum* L.) on morphology, phenolic compound production, gene expression and related drought stress responses, they reported that UV-B reduced the length of stalk, stalk dry weight and number of flower primordia in pepper. Rajashekara et al. (2021) Ultraviolet- In their study examining the effect of C radiation on the genetics and biochemical composition of capsicum plants, they reported that they found minimum amounts of protein, carbohydrates and free fatty acids in plants with very high mutation rates. Lee et al. (2014) used UV-ABC rays to study the mutated lettuce (*Lactuca sativa* L.) growth and phenolic compounds, they reported that repeated types of UV light increased the phenolics in the plant; however, it seriously inhibited the growth of lettuce.

By following these positive developments in mutation breeding, this study aimed to determine the germination rates and the "Effective Dose of Mutation (EMD_{50})" by using two different mutagens (gamma ray and Ethyl Metan Sulfonate chemical) applying to three different pepper varieties (Çermik genotype, Üçburun and Kandil).

MATERIALS AND METHODS

Materials

The plant material used in the study were Çermik pepper, Üçburun and Kandil bell pepper. The seeds of these material used as propagation material. Çermik is a local genotype and grown in Çermik district and Diyararbakır. This genotype also has geographical indication registration certificate and the seeds were taken from Diyarbakır Agricultural Research Institute which selected in recent breeding programme. Üçburun and Kandil bell pepper varieties were also purchased in sealed packages from the authorized dealer of Antalya-based company.

Ethyl Methane Sulfonate as a chemical mutation material supplied from the company of Sigma-Aldrich. Co^{60} source (power 2.07 kGy/h) from physical mutations was irradiated at TENMAK institution.

Methods

This study was carried out in the laboratory and greenhouse of the Department of Horticulture, Faculty of Agriculture in Dicle University. The seeds used in the experiment were sown in laboratory and in greenhouse conditions.

Chemical teratments

The seeds of the plant materials used in chemical trial were kept in containers filled with tap water for 12 hours before EMS application. Different doses of EMS (Lambat et al., 2012; Devi and Selvakumar, 2013; Pharmawati et al., 2018; Soyam, 2021) were prepared (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% and 1%) and 500 seeds were put into each glass beaker; the seeds of the control group (0 dose) were also placed in glass beaker filled with tap water again. For a total of 6 hours and 9 hours, petries with closed lids were shaken for 5 minutes/ hour for getting homogeneous treatment. At the end of 6 and 9 hours (Tantray et al., 2017; Akalp and Pirinç, 2024), these seeds were washed thoroughly under tap water three times at 20 minute intervals and then sown in petries.

Physical teratment

The seeds, which were exposed to gamma radiation by the authorities at the TENMAK office affiliated to the Turkish Atomic Energy Agency, were sown when they arrived at Dicle University in the evening of the same day. For gamma irradiation, 8 different doses of 0, 50, 100, 200, 300, 400, 500 and 600 Gy were applied, recent studies used Co^{60} source (Hayward et al., 1993; Tepe et al., 2003). The seeds exposed to mutagens and in control group were sown in 45’ viols with a 2:1 (peat:perlite) mixture. After all sowing were completed, greenhouse temperatures were kept at 25 ºC in day and 22 ºC at night.

Establishment of experiment

The experiment was set up with 5 replications for each mutation application and 45 seedlings were used in each replication. For germination tests, the experiment was set up with 4 replications in petri dishes in plant growth chamber and each replication included 100 seeds. The germinated seeds were counted on the 7th, 14th, 21st, 28th and 35th days after sowing. Seeds having 2 mm radicle were accepted as germinated. Germination rates were determined with the number of seeds counted on the last day (35th day). 45 days after sowing, seedlings were taken out of the viols and washed under tap water to determine the effective dose of mutation. In the experiments some measurements and observations were taken such as germination percentages of EMS and gamma rays, height of seedlings, for each variety and applications.

The obtained data were analyzed using variance analysis in the IBM SPSS v25 (2024) statistical package program factorial experimental design and linear regression analysis were done with Excel Microsoft program.

RESULTS AND DISCUSSION

After the application of EMS doses on the seeds of three pepper varieties for different periods, germinated seeds counted and the statistical results were given in Table 4. According to the Table 4, germination rates were decreased in all pepper varieties while compared with control group. The effect of EMS applied at different doses for 6 hours and 9 hours on the germination rates of seeds of Kandil variety were statistically significant. While the highest germination rate of 86%; was obtained in control group but the lowest germination rate was O with the dose of 1% for 9 hours application. Similar result were also for Üçburun variety; while the highest germination rate was obtained in control group with 77%, but the lowest germination rate of (O) obtained in 1% doses.
Çermik variety even though being a local genotype have highest germination rate of 92% in the seeds of control group and the lowest germination rate was 29% in 9 hours applications of 1%.

Table 4. Germination percentages of seeds 35 days after EMS application

	KANDİL		ÜÇBURUN		CERMIK	
	6 HOURS	9 HOURS	6 HOURS	9 HOURS	6 HOURS	9 HOURS
Applications	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)
Control	80.50±3.79 a	85.50±2.31 a	74.00±1.37 a	77.00±2.91 a	91.50±1.65 a	90.50±2.90 a
0.1%	79.00±3.53 a	59.00±1.19 a	71.50±2.07 ab	57.50±0.40 b	88.00±0.87 ab	88.50±4.52 a
0.2%	77.00±2.41 a	56.50±3.90 ab	70.00±1.74 abc	54.50±1.24 b	91.00±0.58 a	88.00±0.43 a
0.3%	63.00±5.93 ab	43.50±3.77 bc	62.50±2.15 bc	52.00±0.77 b	87.00±1.04 ab	87.50±5.00 a
0.4%	44.50±2.58 bc	39.00±2.69 cd	58.50±1.46 cd	36.00±0.59 c	81.00±0.58 bc	86.50±2.20 a
0.5%	36.50±4.14 cd	28.50±4.01 d	56.00±2.52 cd	31.50±3.89 c	78.50±1.02 c	86.00±2.33 a
0.75%	33.50±4.52 cd	0.50±0.80 e	56.00±1.02 d	0.00±0.83 d	69.00±3.27 d	35.50±1.72 b
1.0%	22.00±4.12 d	0.00±1.24 e	41.50±7.16 e	0.00±0.83 d	55.00±2.12 e	29.00±2.23 b
p value	<,0001	<,0001	<,0001	<,0001	<,0001	<,0001
CV	15.75	16.23	12.38	10.31	4.37	8.68
LSD	33.29	35.3	32.13	37.86	17.72	36.06

The effect of different doses of gamma rays on germination rate for the seeds of three different pepper varieties were statistically significant as shown in Table 5. As the dose of gamma rays increased, the germination rate were decreased for all three groups. While the highest germination rate of 93% was obtained in control group of Kandil variety, the lowest germination rate of 9.50% was obtained at 600 Gy dose. A similar result was observed in Üçburun variety; while the highest rate (93.50%) was obtained in the control group, but 600 Gy dose have the lowest germination rate of 16%. In Çermik genotype the difficulties in germination can be explained, by incerasing of dose cause to decrease the germination rate. As the highest germination rate was obtained in the seeds of control group (94%), the lowest germination rate (47%) obtained at the highest dose of 600 Gy.

As a result of the study it can be said that decreases in germination percentages as a result of increasing EMS application duration and EMS doses. A similar situation also occurred after the use of gamma rays, which are physical mutagens. As the doses of gamma rays increase, the germination rate decreases. Pharmawati et al. (2018) reported; using 0.5%, 0.75% and 1.0% EMS doses on pepper the germination and emergence of seeds decreased with increasing dose while comparing to control group.

Similar results were reported by Sood et al. (2016) in determining the lethal dose with gamma rays and EMS application on bell pepper. In the study, it was reported that high doses of gamma rays (19kR and 22kR) and EMS (2.0 % and 3.0%) caused a decrease in percentages of seed germination and less growth of seedlings. Sanjai Gandhi et al. (2014) reported that germination percentages decreased with increasing doses using ethyl methane sulfonate (10, 20, 30, 40 and 50 mM) and diethyl sulfate (5, 10, 15, 20 and 25 mM) mutagens. Arisha et al. (2014) reported that germination percentages decreased with increasing doses of EMS applied to pepper seeds (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50% v/v). Alcantara et al. (1996) tried to determine seed mutagenesis in pepper using 0.5%, 1.0% and 1.5% EMS doses and seeds exposed to 5°C, 10°C, 15°C and 20°C for 3, 6 and 9 hours.

They stated that seeds treated with 1.5% EMS for 9 hours at 20°C in the M1 generation had the lowest germination percentage among 36 treatments. Jabeen and Mirza (2002) reported that germination decreased with increasing EMS (0.01, O.1, O.5 % VN) concentrations in 3 and 6 hour applications in order to increase genetic variations in pepper. It was reported that the lowest germination was obtained with 0.5% EMS application for 6 hours. Saba and Mirza (2002) tried to determine genetic variations in pepper by using 0.1%, 0.5% and 1% ethyl methane sulfonate (EMS) doses for 3 and 6 hours. It was reported that germination rates decreased with increasing doses.

Table 5. Germination percentages of seeds 35 days after gamma ray application.

	KANDİL	ÜÇBURUN	CERMIK
Applications	Germination rate (%)	Germination rate (%)	Germination rate (%)
Control	93.00±1.58 a	93.50±0.95 a	93.50±1.18 a
50	81.50±0.54 b	79.75±1.58 b	87.00±0.72 b
100	80.00±0.60 b	74.25±0.69 c	86.50±0.24 b
200	75.00±0.72 bc	72.75±0.58 cd	84.50±0.24 b
300	71.00±1.97 c	68.50±0.98 d	79.25±0.74 c
400	49.50±1.59 d	51.00±1.73 e	75.00±0.87 c
500	16.00±1.95 e	19.50±0.20 f	55.75±0.48 d
600	9.50±1.47 e	16.00±0.70 f	46.75±1.59 e
CV	5.10	3.75	2.45
p value	<,0001	<,0001	<,0001
LSD	42.98	38.72	22.52

The effect of physical mutation (gamma) doses applied to different types of pepper seeds on plant height during the seedling period is given in Table 6. According to the table, the effect of gamma ray application on seedlings height was statistically significant for all three types of pepper seeds.

Table 6. Effect of different Gamma doses on seedling height.

	KANDİL	ÜÇBURUN	CERMIK
Applications		Seedling height (cm)	
Control	10.71±0.22 a	9.19±0.14 b	12.60±0.46 a
50	9.10±0.22 b	8.09±0.13 c	10.15±0.32 b
100	6.05±0.13 c	7.15±0.13 c	8.18±0.22 c
200	4.97±0.07 d	6.94±0.13d	6.68±0.21 d
300	4.09±0.07 e	5.93±0.13e	6.76±0.30 cd
400	4.00±0.07 e	4.85±0.22 ef	4.76±0.24 e
500	3.91±0.07 e	4.08±0.22 f	4.00±0.32e
600	3.79±0.07 e	3.68±0.22 a	3.39±0.29 e
p value	<,0001	<,0001	<,0001
CV	9.420976	11,2007	14.62497
LSD	4.71	3.59	5.4

In terms of plant height, which is the most important parameter in determining the effective mutation dose in mutation breeding, after gamma irradiation, the highest height was found in control group in Kandil, Üçburun and Çermik pepper seedlings respectively; 10,71 cm, 9,19 cm and 12,60 cm. As the doses increased, decreases were seen in these values compared to the seedlings of control group (Kandil: 3,79 cm; Üçburun: 3,68 cm; Çermik: 3,39 cm). According to the results of the statistical analysis; EMD_{50} dose was calculated as 315,49 Gy in Kandil variety as a result of gamma application. EMD_{50} of the Kandil variety is given in figure 1.

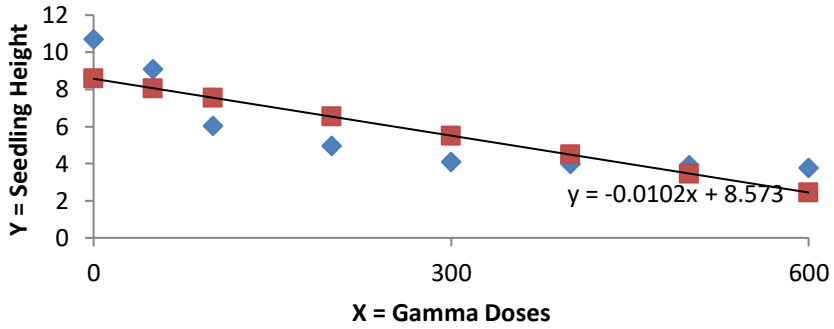

Figure 1. Change in seedling heights of Kandil variety as a result of the application of gamma doses

The figure showing the EMD_{50} as a result of applying different doses of Gamma to the ÜB variety is given below (Figure 2). The EMD_{50} dose in the ÜB variety was determined as 454.95 Gy.

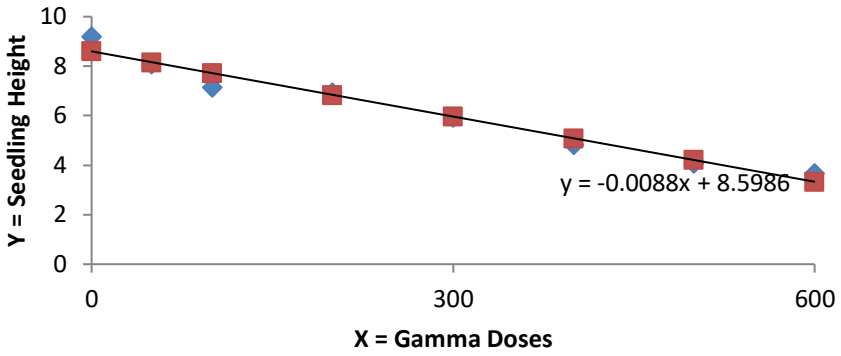

Figure 2. Change in seedling heights of Üçburun variety as a result of the application of gamma doses

In Çermik genotype, the EMD_{50} dose was determined by detecting abnormal shortening in the seedlings as a result of the application of Gamma rays. In Çermik genotype, EMD_{50} was determined 329.26 Gy due to Gamma irradiation (Figure 3).

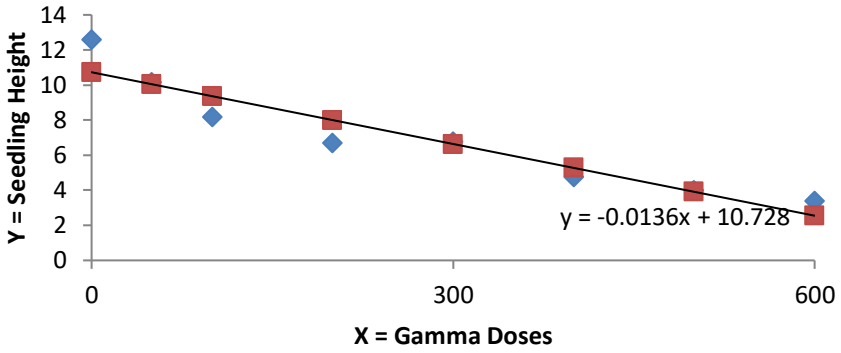

Figure 3. Change in seedling heights of Çermik variety as a result of the application of gamma doses

As mentioned in the studies, there are different mutation doses among vegetable species and varieties. Kantoğlu (2022) reported that there were decreases in plant height with increasing gamma doses in his study to determine the EMD_{50} dose in watermelon. In addition, Puripunyavanich, (2003), Velkov et al., (2016); Ernest et al., (2020), Çelik et al., (2021), Kantoğlu and Kunter, (2021), Zafar et al., (2022) reported decreases in seedling heights in parallel with increasing radiation doses in their studies. Tepe et al., (2003) reported that shoot development decreased at doses of 200 Gy and that there were especially significant decreases at the dose of 400 Gy to find the effective mutation dose in pepper. Micke and Donini (1993) also stated that doses of 140-220 Gy reduced shoot development. Sood et al., (2016) reported that seedlings'length decreased in bell pepper at high doses of gamma rays (19 kR and 22 kR). Kökpınar et al., (2024) reported that for lettuce, plant height decreased as the dose of gamma rays increased and the decreases were especially evident at the dose of 200 Gy.

The parameters shows the effects of different EMS doses on three different pepper seedlings were given in Table 7. When table is examined, it was determined by examining the results the use of EMS doses in pepper mutation breeding is important.

Table 7. Effect of Different EMS Doses Applied at Different Times on Seedling height

	Kandil	ÜB	Cermik	Kandil	ÜB	Cermik
	6 Hours			9 Hours		
Applications	Seedling height (cm)			Seedling height (cm)		
Control	13.53±0.18 a	10,29±0,25 a	14,65±0,57 a	13.61±0.11 a	11.46±0.27 a	13.75±0.43 a
0.1%	10.49±0.19 b	9,63±0,16 ab	14,11±0,58 a	9.69±0.13 b	9.58±0.13 b	13.22±0.36 a
0.2%	10.09±0.13 b	9,01±0,13 bc	12,58±0,54 ab	8.65±0.15 c	8.83±0.08 b	11.70±0.34 b
0.3%	9.15±0.12 c	8,44±0,19 cd	12,59±0,53 ab	7.75±0.13d	7.79±0.20 c	11.68±0.40 b
0.4%	7.65±0.14d	8,14±0,17 cd	11,58±0,45 bc	7.34±0.16 de	7.69±0.23 c	9.90±0.23 c
0.5%	7.23±0.18 d	7,55±0,32 de	11,65±0,41 bc	6.86±0.19 e	6.95±0.18 cd	9.49±0.20 cd
0.75%	5.46±0.09 e	6,91±0,16 e	9,83±0,24 cd	4.91±0.10 f	6.62±0.15d	8.29±0.28 d
1.0%	4.34±0.09 f	4,90±0,22 f	7,84±0,20 d	3.88±0.10g	4.63±0.27 e	6.45±0.18 e
p value	<,0001	<,0001	<,0001	<,0001	<,0001	<,0001
CV	8.21	8,62	13,20	8.34	8.44	10.08
LSD	4.71	3,59	4,35	5.53	0.84	4.39

The differences in plant heights was statistically significant as a result of application of EMS doses and different durations. In Kandil, Üçburun and Çermik pepper seedlings, the highest height was determined in control group seedlings (6-hour application: Kandil: 13,53 cm, Üçburun: 10,29 cm and Çermik: 14,65 cm; and 9-hour application: Kandil: 13,61 cm, Üçburun: 11,46 cm and Çermik: 13,75 cm); as the doses increased, decreases were obtained in these values compared to the seedlings in the control group (the lowest heights were at 1.0% doses). It has also determined in recent studies; there were shortenings in plant heights measured after the application of EMS in pepper (Kantoğlu et al., 2014; Pharmawati et al., 2018; Akalp and Pirinç, 2024). Pharmawati et al. (2018) reported that as a result of the application of ethyl methane sulfonate (0,5%, 0,75% and 1,0% EMS) in pepper, plants were stunted in the M2 generation. Sood et al., (2016) tried to determine the LM_{50} dose using gamma rays and EMS in bell pepper. They reported that plant heights decreased with the application of 2,0% and 3,0% EMS. Jabeen and Mirza (2002) reported that the 0.5% dose reduced height of seedlings by using different doses of EMS (0,01, 0,1, 0,5% VN) in pepper.

The effect of EMS on seedling height in three different pepper types was tried to determine as EMD_{50} value. According to the measurements on the seedlings after 6 hours of application in Kandil variety, the EMD_{50} value was determined as 0,67%. The figure below shows the effective mutation dose for Kandil variety (Figure 4).

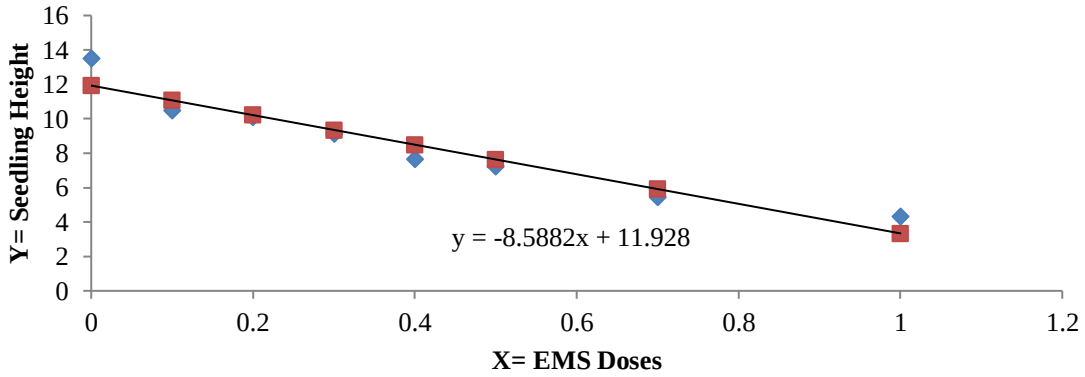

Figure 4: Determination of EMD_{50} in Kandil pepper variety after 6 hours of EMS application

The EMD_{50} determined as a result of applying different EMS doses for 6 hours to Üçburun variety is given in figure 5. According to the data obtained by measuring the height of ÜB seedlings, EMD_{50} was determined as 0.97%.

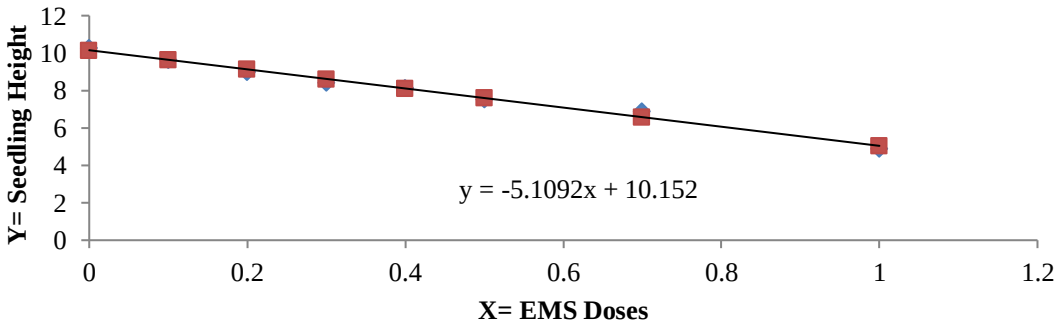

Figure 5. Determination of EMD_{50} after 6-hour EMS application in Üçburun pepper variety

The EMD_{50} determined as a result of applying different EMS doses for 6 hours in Çermik pepper genotype was given in figure 6. According to the data obtained by measuring the height of Çermik seedlings, EMD_{50} was determined as 1,08%.

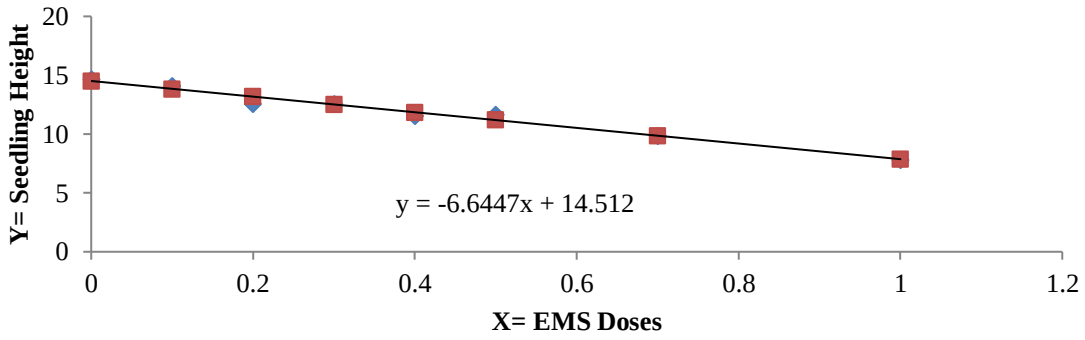

Figure 6: Determination of EMD_{50} in Cermik pepper genotype after 6-hour EMS application.

In Kandil pepper variety, the EMD_{50} value was determined as 0,52% according to the measurements of the seedlings after 9 hours of application. The figure below shows the effective mutation dose in Kandil variety (Figure 7).

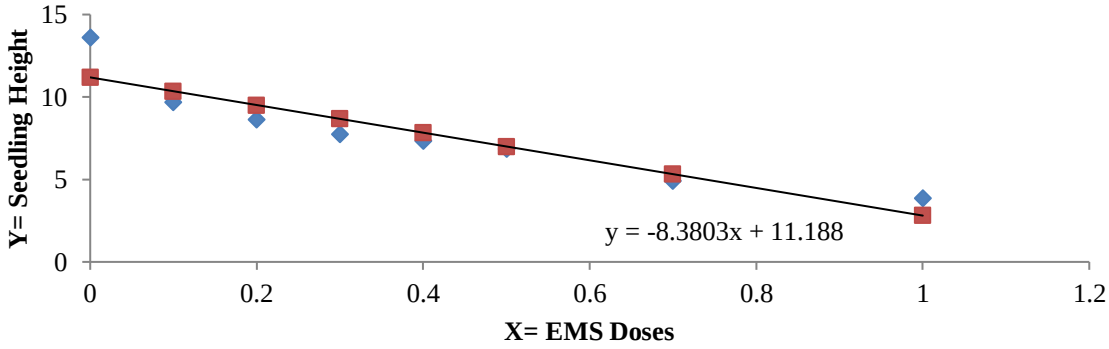

Figure 7. Determination of EMD_{50} after 9-hour EMS application in Kandil pepper variety.

The EMD_{50} determined as a result of applying different EMS doses for 9 hours to Üçburun pepper variety was given in figure 8. According to the data obtained by measuring the height of Üçburun seedlings, EMD_{50} was determined as 0,77%.

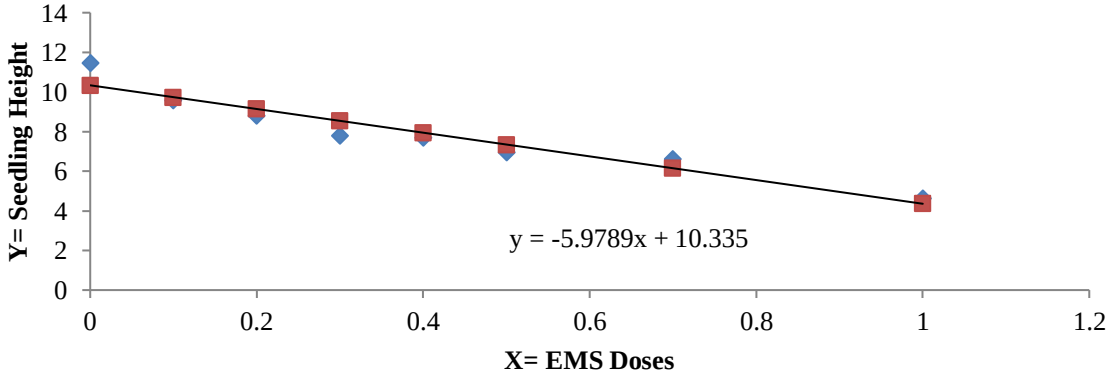

Figure 8: Determination of EMD_{50} after 6-hour EMS application in Üçburun pepper variety.

The EMD_{50} determined as a result of applying different EMS doses for 9 hours in Çermik pepper genotype was given in figure 9. According to the data obtained by measuring the height of Çermik seedlings, EMD_{50} was determined as 0,89%.

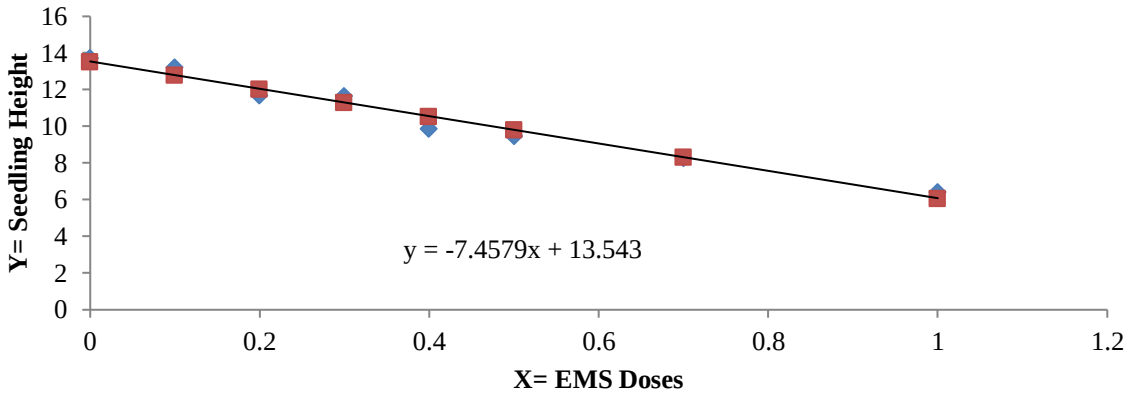

Figure 9: Determination of EMD_{50} in Cermik pepper genotype after 9 hours of EMS application.

CONCLUSION

As the doses of physical and chemical mutagens, which are among the mutation breeding methods, increase, they create physiological and morphological changes in the plant. Mutation breeding methods, which plant breeders frequently use to create variations in shorter time with lower cost while compared to other method. And also provide the opportunity to create new variety candidates with desired characters in many plant species. At the end of the study, EMD_{50} in three different pepper genotypes as a result of CO^{60} irradiation, one of the gamma ray types, was Kandil: 315.49; for Üçburun: 454.95 and Çermik: 329.26 Gy. The results obtained from Kandil bell and Üçburun pepper varieties, which respond to the lower and upper limits of gamma ray dose, gave similar results to the doses applied to other commercial varieties in academic studies. In the study, the application of different EMS doses gave similar results for three different pepper seeds. At the end of the study, it was observed that with increasing EMS doses, there were decreases in the "plant height" parameter, which is used as a reference parameter in determining the effective mutation dose. The results of this study were parallel to other academic studies were obtained, with different doses of EMS applied to three different pepper seeds, the EMD_{50} value in pepper was between 0,67% and 1,08% in 6 hours of application. And It was found between 0,52% and 0,89% for 9 hours of application. It was determined that the mutation frequency increased with the increase in application times; but it was determined that the EMD_{50} value also decreased.

In this study, in which different physical and chemical mutagens were used, applications were made to three different pepper varities, the effect of mutagen doses on seed germination and pepper seedlings were investigated, and results of this research will be reference to future studies on mutation breeding. The later level of this study will serve new breeding lines for new varieties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

There is no conflict of interest in the article

Author contribution

Each author has the equal contributiıon (for each 50%)

Ethics committee approval

There is no need for ethichs committee approval.

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Morphological and pomological characterization of F_2 generation cucumber (*Cucumis sativus* L.) plants of different fruit types

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Abstract

In general, when the traits related to cucumber breeding are examined, morphological traits such as leaf and flower characteristics, fruit; size, shape, spines, fruit flesh set, as well as yield are among the most important factors among the breeding selection criteria. The morphological and pomological characterization of 109 plants belonging to 16 F_2 lines of different fruit types under soilless agriculture conditions were carried out and lines with breeding material value were identified. The average fruit weights of the lines with different fruit types were determined as mini (snack) type 53.88 g, beith alpha type 138.84 g, gherkin type 49.95 g and long european type 194.22 g. The highest fruit flesh firmness was determined as 0.98 kg/cm^2 in lines with mini (snack) fruit type, while the lowest was determined as 0.59 kg/cm^2 in lines with beith alpha fruit type. Warts on the fruit surface were detected on C355 and N285 lines with gherkin fruit type and C348 line with long european fruit type, while there were without warts on the fruit surfaces of other lines. Beith alpha fruit type, 8 plants of line C350 had monoecious flower structure, while the plants of other lines had gynoic flower structure. The longest internode was 13.75 cm in line N285 and the shortest internode was 8.53 cm in line C350. Differences between lines and plants with different fruit type were determined by principal component analysis. It was determined that there was a wide variation among the plants in terms of all the traits examined and the traits that can be used as breeding material for future studies in cucumber were identified.

Keywords: Fruit type, Selection, Warts, Internode, Variation

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INTRODUCTION

Cucumber is a species of vegetable belonging to the *Cucurbitaceae* family, which is widely cultivated in the world. Cucumber (*Cucumis sativus* L.), whose origin is India, is one of the oldest cultivated vegetable species. Cucumber can be cultivated in tropical and subtropical climatic conditions as well as temperate climatic conditions in terms of climate requirements. Cucumber fruits are fragrant and delicious with a wealth of nutrients that can be consumed fresh, cooked or pickled (Mercke et al., 2004; Zhang et al., 2021). In regions with cold climatic conditions, it is cultivated in open or under greenhouse during the summer months (Robinson & Decker-Walters, 1997; Salcedo et al., 2018). In the world, approximately 94 million tons of cucumber is produced on 2.174.347 ha of land, with China ranking first with 77.258.256 tons, followed by Türkiye (1.938.545 tons) and Russia (1.635.903 tons) (FAO, 2022). Cucumber has an important place in human nutrition. Cucumber regulates the human body's fluid intake, blood pressure and sugar and fat repair, soothes the skin, aids digestion and aids weight loss. Cucumbers contain plenty of potassium, magnesium, manganese and high levels of vitamins A, C and K (Chakraborty & Rayalu, 2021). The immature fruits of cucumber are used fresh or for gherkins. The fruits of the cucumber plant are harvested about two weeks after flowering, depending on the variety. At this stage, the whole fruit is consumed because the fruit texture is crunchy and the seeds are small and not fully ripe (Chakraborty & Rayalu, 2021). Unlike fruits eaten ripe, where metabolic traits such as sweetness, flavor and aroma are the main

quality determinants, the predominant morphological traits that determine cucumber fruit quality are size and shape, external traits such as wax, spines and warts, and internal traits such as flesh thickness and seed cavity size. These morphological characteristics are evident in the different cucumber cultivars grown around the world. East Asian cucumbers consumed fresh are typically North Chinese type (Langa type), North American cucumber types intended for fresh markets are characterized by smooth, medium length (20-30 cm), while North American and European pickling cucumbers have spiny and short fruits (5-15 cm). Beit Alpha or Mediterranean cucumbers have a parthenocarpic structure and thin skin thickness. In parthenocarpic cucumber varieties, fruit formation occurs without pollination. In addition to the dominant market classes, there is a wide diversity in fruit morphology in cucumber genetic material (Grumet et al., 2022). Fruit size is related to both cell number and a combination of cell size. In cucumber fruits, cell number is mainly formed during ovary development, with the second stage of cell division occurring during the first 4-5 days after pollination. Fruit shape is typically elongated and cylindrical in commercially grown cucumbers. However, cucumber fruit vary greatly in several factors that influence shape, including length, diameter, uniformity (cylindrical and conical) and tendency to curl (Wei et al., 2016; Zhu et al., 2016; Gao et al., 2020). The surface shape of cucumber fruits varies according to the presence of the waxy layer, the number, size and shape of ridges and spines. The presence of spines on the fruit surface is a hydrophobic layer that covers the aerial surfaces of plants to limit cuticular water loss; provide mechanical support for fruit growth and development; and protect against environmental stresses such as pathogens, insects, UV radiation and drought (Yang et al., 2014; Grumet et al., 2022). The rind of ripe cucumber fruit can be of various colors such as white-green-yellow-orange-brown. However, the rind color of immature cucumber fruit at the harvest stage has a narrower spectrum than other immature fruits, ranging from white to light green to dark green. Variation in skin color of immature fruit can be a desirable novelty for the market. In fruit flesh color, a narrower range of color is observed in the fruit mesocarp and endocarp. Cucumber fruit flesh is typically white, but can also have yellow, orange and green flesh color. The yellow and orange flesh color is due to the accumulation of carotenoids, including beta-carotene (Zhu et al., 2016; Wang et al., 2020; Grumet et al., 2022). The development of high-yielding varieties with better fruit quality is the main objective of cucumber breeding programs worldwide. The spread of parthenocarpic cucumber varieties in our country and in the world increases cucumber production. Türkiye is rich in plant biodiversity and is a production center for many vegetable species. However, since the origin of cucumber is not Türkiye, the cucumber varieties used in our country were first originated abroad. This process continued with breeding companies bringing new varieties to our country, and then breeding companies in our country developed new hybrid or standard varieties using these varieties. Although there are studies on the characterization of hybrid or local cucumber varieties in our country, there is a need for much more studies. Characterization studies are very important for breeding studies in terms of yield, disease resistance and determination of cucumber types suitable for market demand. In addition, a good identification of the starting material of breeding programs provides great advantages by preventing time and economic loss in the breeding process. Therefore, it is necessary to determine the agronomic and genetic performance of these genetic materials as well as their characterization. The aim of this study was to make a morphological and pomological characterization of 16 F_2 cucumber lines and to select lines with breeding material value.

MATERIALS AND METHODS

Plant Material

As plant material, F_2 plants obtained by selfing in 2023 from cucumber cultivars of different fruit types, which are widely used in greenhouse cultivation in Türkiye, were used.

Method

The morphological and pomological characterization study was carried out in 2024 in the geothermally heated, venlo type, glass and fully automated R&D greenhouse of Kırşehir Ahi Evran University. Seed sowing was carried out in a 128-cell seedling plug tray filled with a peat:perlite mixture at a 3:1 ratio. Plants were grown in the greenhouse by irrigation and fertilization until the first true leaf stage. When the seedlings reached planting size, they were planted in cocopeat medium with a distance of 25 cm between rows and 100 cm between rows. In the experiment, the number of plants from each F_2 line specified in Table 1 was transplanted. Irrigation, fertigation and acclimatization processes (the amount of water and fertilizer was adjusted depending on the plant growth stage and greenhouse temperature) were carried out with an automation system. Since the plants were in F_2 generation, the experiment was not set up with replicates. The averages of the measurements and observations were determined according to the number of F_2 plants within the lines.

Examined Parameters

In the experiment, morphological and pomological characterization was carried out in terms of plant and fruit traits according to IPGRI's description list for cucumber and UPOV criteria (Protocol for tests of difference, uniformity and stability, UPOV TG/44/11 Rev.3). The 7 morphologically examined criteria and their criteria are given in Table 2. Fruit measurements were completed when the fruits in the center of the plants were ripe. During the observation and measurement period of the study, the length of the traits to be examined were measured with a ruler, diameter and thickness were measured with calipers, and fruit weight was measured with a balance. Fruit

juice Ec and pH values were measured with Ec meter and pH meter with Extech device. Fruit flesh firmness was measured with PCEPTR 200 penotrometer. Soluble solids content (SSC) was measured with Hanna HI96801 digital refractometer.

Table 1. F_2 lines and plant numbers used in the study

F_2 Lines	Number of Plants	F_2 Lines	Number of Plants
C323	8	C340	3
C343	8	C350	8
C290	4	C357	8
C333	8	C355	4
C336	7	N285	4
C339	8	C295	8
C304	8	C312	7
C307	8	C348	8
		Total	109

Table 2. Morphologically parameters

No	Observed Characteristics	Scale Values
1	Fruit color	Dark Green, Green, Light Green
2	Number of fruits per node (Fruit set)	Multi, Semi-Multi, Single
3	Fruit spine	Present, Light, Absent
4	Fruit wart	Present Absent
5	Plant growth	Very Strong, Strong, Medium Strong
6	Fruit type	Mini (snack), Beith Alpha, Gherkin, Long European Type (LET)
7	Flower Structure	Monoecious, Gynoic

Statistical analysis

The data obtained in the studies were analyzed by one-way analysis of variance (ANOVA) using SPSS 18.0 statistical software (IBM, Chicago, IL, USA) at 5% significance level and the difference between the means was determined by Duncan multiple comparison test. In addition, principal component analysis (PCA) were performed using Minitab statistical software.

RESULTS

The plants in the F_2 generation obtained by selfing 16 hybrid cucumber cultivars of different types widely used in greenhouse cultivation were classified according to fruit type. In terms of fruit characteristics, the line averages of average fruit weight, fruit diameter, fruit length, fruit flesh firmness, SCC, EC and pH parameters were determined (Table 3). Among 16 lines, 2 of them were identified as mini (snack), 9 as Beith Alpha, 2 as gherkin and 3 as long european fruit type (Figure 1).

Figure 1. Long European (C-312), Beith Alpha (C-333), Mini (snack) (C-323) and Gherkin (C-355) type cucumber fruits

The average fruit weight of 9 cucumber lines of beith alpha type was 138.84 g. The highest average fruit weight among beith alpha lines was determined as line C-339 with 190.70 g. The average fruit weight of 3 cucumber lines of long european type was 194.22 g, while the line with the highest fruit weight was determined as line C-312 with 243.10 g. The average fruit weight, fruit length and fruit diameter of the lines with 2 mini (snack) and 2 gherkin fruit types were lower than the lines with beith alpha and long european fruit types used in the study. The highest average flesh firmness was measured in the lines with mini (snack) (0.98 kg/cm^2) and gherkin (0.78 kg/cm^2) fruit types, while the lowest average flesh firmness was determined in the lines with beith alpha fruit type with 0.59 kg/cm^2. The highest fruit juice SSC was determined in the lines with mini (snack) fruit type (3.95%), while the lowest SSC was determined in the lines with gherkin fruit type (2.77%). The highest juice Ec value was determined in mini (snack) type lines (2.83) and the lowest in long european type lines (2.47). Fruit juice pH of 16 lines ranged between 6.02 (C-290) and 7.05 (C-390). There was no statistically significant difference between the lines in terms of juice EC, pH, and SSC parameters ($p>0.05$) (Table 3).

Table 3. Average fruit weight, fruit length, fruit flesh firmness, fruit juice SSC, Ec and pH of cucumber lines in F_2 generation with different fruit types

F_2 Lines	Fruit Type [mini (snack), beith alpha, gherkin, long european]	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Fruit Flesh Hardness kg/cm^2	Soluble Solids Content (SSC)	Fruit Juice Ec	Fruit Juice pH
C323	Mini (snack)	63.83ef	10.27g	3.17b-e	0.86ab	3.93	2.67	6.12
C343	Mini (snack)	43.93f	9.97g	2.53de	1.09a	3.97	2.98	6.15
Average		53.88	10.12	2.85	0.98	3.95	2.83	6.14
C290	Beith Alpha	121.63b-f	14.50de	3.63a-c	0.67bc	2.73	3.06	6.02
C333	Beith Alpha	128.90b-e	16.57c-e	3.43a-c	0.68bc	3.57	2.57	6.14
C336	Beith Alpha	131.80b-e	15.83c-e	3.50a-c	0.68bc	3.70	2.53	6.07
C339	Beith Alpha	190.70ab	18.50bc	3.90ab	0.46bc	3.10	2.63	6.09
C304	Beith Alpha	96.33d-f	13.83ef	3.33a-d	0.65bc	2.77	3.00	6.11
C307	Beith Alpha	108.80c-f	16.10c-e	3.03c-e	0.51bc	3.17	2.97	6.82
C340	Beith Alpha	170.17a-d	15.33c-e	4.03a	0.36c	2.83	2.87	6.46
C350	Beith Alpha	126.57b-e	16.73c-e	3.50a-c	0.56bc	3.17	2.70	6.05
C357	Beith Alpha	174.70a-c	18.03b-d	4.10a	0.71a-c	3.13	2.59	6.10
Average		138.84	16.16	3.61	0.59	3.13	2.77	6.21
C355	Gherkin	45.10f	10.33fg	2.37e	0.79ab	2.63	2.68	6.32
N285	Gherkin	54.80ef	10.07g	3.00c-e	0.72a-c	2.90	2.46	6.33
Average		49.95	10.20	2.69	0.76	2.77	2.57	6.33
C295	Long European	163.60b-d	21.53b	3.43a-c	0.71a-c	3.57	2.41	6.27
C312	Long European	243.10a	26.67a	3.80a-c	0.73a-c	2.90	2.47	7.05
C348	Long European	175.97a-c	21.33b	3.60a-c	0.80ab	3.50	2.54	6.38
Average		194.22	23.18	3.61	0.75	3.32	2.47	6.57
p value		***	***	***	***	n.s.	n.s.	n.s.

Different letters in the same column indicate that the difference between groups is significant $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Principal component analysis (PCA) was performed to classify the cucumber lines in the F_2 generation based on fruit pomological traits (Figure 2). According to the analysis, two principal components (99.90% according to PC1 and 0.09% according to PC2) accounted for 99.99% of the total variation. The lines with the highest fruit length, fruit diameter, fruit weight and fruit juice pH were located in regions I. and IV. of the graph. The lines with long european and Beith Alpha fruit type were all located in regions I. and IV. of the graph. Cucumber lines with mini (snack) and gherkin fruit type were located in the II. and III. regions of the graph. The lines with the highest flesh firmness and SSC were C-343 and C323 with the mini (snack) fruit type, while the lines with the lowest SSC were the lines with the Beith Alpha fruit type, which were located in region IV. of the graph. The lines with the

highest juice Ec were located in region III. of the graph, while the lines with the lowest fruit juice Ec were located in region I. of the graph.

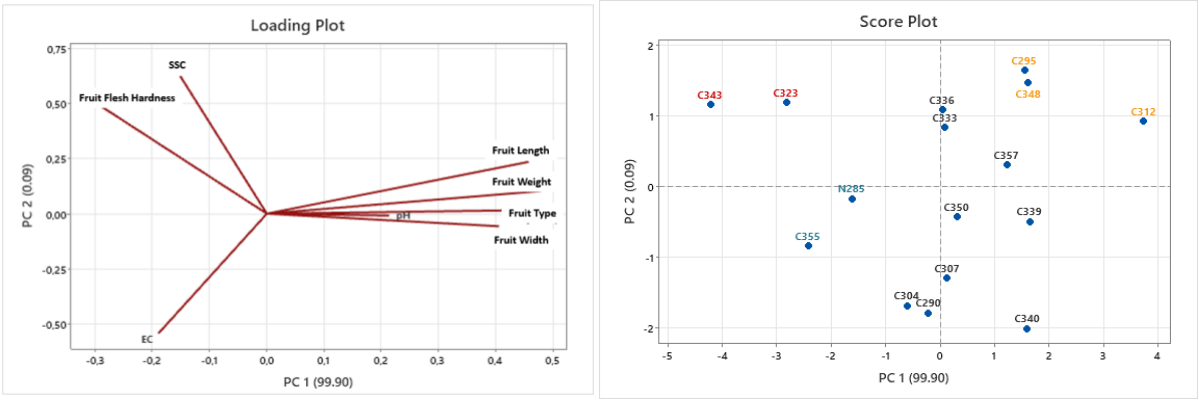

Figure 2. Principal component analysis (PCA) of the average fruit weight, fruit length, fruit flesh firmness, fruit juice SSC, Ec and pH parameters of F_2 cucumber lines with different fruit types

As a result of the parameters examined, in terms of fruit color, all plants of the silor fruit type, line C-313 had dark green fruit color, while the plants of line C-343 had green fruit color. F_2 plants of line C-323 showed single, semi-multi and single fruit set, while plants of line C-343 had multi and semi-multi fruit set. While the fruits of 16 F_2 plants of both lines were spineless, they had strong plant vigor and gynoic flower structure. The difference between the mean internode length of the F_2 plants of line C-323 and the mean internode length of the plants of line C-343 was 1.12 cm, while this difference was 2.44 cm in leaf width and 0.45 cm in leaf length (Table 4).

Table 4. Fruit color, fruit set, fruit spine, fruit wart, plant vigor, flower structure, internode length, leaf width and length of mini (snack) type lines

F_2 Code	Fruit Color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C323-1	Dark Green	Single	Absent	Absent	Strong	Gynoic	10.00	28.00	25.50
C323-2	Dark Green	Multi	Absent	Absent	Strong	Gynoic	11.00	30.50	28.00
C323-3	Dark Green	Multi	Absent	Absent	Strong	Gynoic	10.00	23.50	22.50
C323-4	Dark Green	Semi Multi	Absent	Absent	Strong	Gynoic	10.00	30.00	27.00
C323-5	Dark Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	28.50	26.50
C323-6	Dark Green	Single	Absent	Absent	Strong	Gynoic	12.00	29.50	26.00
C323-7	Dark Green	Semi Multi	Absent	Absent	Strong	Gynoic	12.00	25.50	23.50
C323-8	Dark Green	Semi Multi	Absent	Absent	Strong	Gynoic	12.00	24.50	22.50
Average							11.00	27.50	25.19
C343-1	Green	Multi	Absent	Absent	Strong	Gynoic	12.00	31.50	24.00
C343-2	Green	Multi	Absent	Absent	Strong	Gynoic	9.00	27.00	26.00
C343-3	Green	Semi Multi	Absent	Absent	Strong	Gynoic	9.00	29.50	26.00
C343-4	Green	Semi Multi	Absent	Absent	Strong	Gynoic	9.00	36.50	27.00
C343-5	Green	Multi	Absent	Absent	Strong	Gynoic	10.00	34.20	25.50
C343-6	Green	Multi	Absent	Absent	Strong	Gynoic	11.00	27.00	27.00
C343-7	Green	Semi Multi	Absent	Absent	Strong	Gynoic	9.00	27.50	24.50
C343-8	Green	Multi	Absent	Absent	Strong	Gynoic	10.00	26.50	25.00
Average							9.88	29.96	25.63

When the fruit color of 9 F_2 lines of Beith Alpha type was evaluated; 3 lines (C-290, C-340, C-357) had dark green, 4 lines (C-333, C-336, C-339, C-307) had green and 2 lines (C-304, C-350) had light green fruit color. While all F_2 plants of line C-339 showed multi fruit set, plants of line C-307 had semi-multi fruit set and all plants of line C-340 had single fruit set. The lines were uniform in terms of fruit shape, plant vigor and flower structure. In terms of flower structure, except for the F_2 plants belonging to the C-350 line, all other F_2 plants had gynoic flower structure. Among the Beith Alpha type lines, the highest average internode length and leaf width were determined in line C-357 with 11.63 cm and 31.09 cm, respectively, while line C-340 had the highest average leaf length with 26.17 cm (Table 5).

Table 5. Fruit color, fruit set, fruit spine, fruit wart, plant vigor, flower structure, internode length, leaf width and length of Beith Alpha type lines

F_2 Code	Fruit Color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C290-1	Dark Green	Single	Absent	Absent	Medium Strong	Gynoic	10.00	28.00	24.50
C290-2	Dark Green	Semi Multi	Absent	Absent	Medium Strong	Gynoic	9.00	29.30	24.50
C290-3	Dark Green	Semi Multi	Absent	Absent	Medium Strong	Gynoic	10.00	26.80	24.00
C290-4	Dark Green	Semi Multi	Absent	Absent	Medium Strong	Gynoic	8.00	27.50	24.00
Average							9.25	27.90	24.25
C333-1	Green	Semi Multi	Absent	Absent	Very Strong	Gynoic	8.00	26.00	23.00
C333-2	Green	Semi Multi	Absent	Absent	Very Strong	Gynoic	10.00	28.00	23.00
C333-3	Green	Semi Multi	Absent	Absent	Very Strong	Gynoic	10.00	23.70	21.10
C333-4	Green	Semi Multi	Absent	Absent	Very Strong	Gynoic	8.00	26.00	22.70
C333-5	Green	Single	Absent	Absent	Very Strong	Gynoic	8.00	21.20	19.80
C333-6	Green	Semi Multi	Absent	Absent	Very Strong	Gynoic	10.00	23.90	21.60
C333-7	Green	Single	Absent	Absent	Very Strong	Gynoic	7.00	26.40	20.00
C333-8	Green	Single	Absent	Absent	Very Strong	Gynoic	9.00	25.00	22.00
Average							8.75	25.03	21.65
C336-1	Green	Multi	Light	Absent	Medium Strong	Gynoic	11.00	26.00	24.50
C336-2	Green	Multi	Light	Absent	Medium Strong	Gynoic	12.00	29.50	25.50
C336-3	Green	Multi	Light	Absent	Medium Strong	Gynoic	10.00	23.50	24.00
C336-4	Green	Semi Multi	Light	Absent	Medium Strong	Gynoic	8.00	24.50	23.00
C336-5	Green	Multi	Light	Absent	Medium Strong	Gynoic	11.00	26.50	26.50
C336-6	Green	Semi Multi	Light	Absent	Medium Strong	Gynoic	9.00	31.00	25.00
C336-7	Green	Semi Multi	Light	Absent	Medium Strong	Gynoic	13.00	32.00	28.50
Average							10.57	27.57	25.29
C339-1	Green	Multi	Light	Absent	Strong	Gynoic	11.00	25.00	23.00
C339-2	Green	Multi	Light	Absent	Strong	Gynoic	11.00	25.50	23.50
C339-3	Green	Multi	Light	Absent	Strong	Gynoic	11.00	25.00	21.50
C339-4	Green	Multi	Light	Absent	Strong	Gynoic	10.00	26.50	25.00
C339-5	Green	Multi	Light	Absent	Strong	Gynoic	9.00	22.50	28.80
C339-6	Green	Multi	Light	Absent	Strong	Gynoic	9.00	25.50	27.00
C339-7	Green	Multi	Light	Absent	Strong	Gynoic	9.00	28.00	24.50
C339-8	Green	Multi	Light	Absent	Strong	Gynoic	9.00	32.00	20.20
Average							9.88	26.25	24.19

F_2 Code	Fruit Color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C304-1	Light Green	Single	Absent	Absent	Strong	Gynoic	12.00	24.50	21.00
C304-2	Light Green	Semi Multi	Absent	Absent	Strong	Gynoic	9.00	29.00	24.50
C304-3	Light Green	Semi Multi	Absent	Absent	Strong	Gynoic	8.00	33.00	28.00
C304-4	Light Green	Single	Absent	Absent	Strong	Gynoic	9.00	31.00	27.00
C304-5	Light Green	Semi Multi	Absent	Absent	Strong	Gynoic	8.00	21.50	21.00
C304-6	Light Green	Single	Absent	Absent	Strong	Gynoic	8.00	29.00	27.00
C304-7	Light Green	Semi Multi	Absent	Absent	Strong	Gynoic	8.00	28.50	26.00
C304-8	Light Green	Semi Multi	Absent	Absent	Strong	Gynoic	8.00	29.70	27.50
Average							8.75	28.28	25.25
C307-1	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	31.00	27.00
C307-2	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	30.70	27.00
C307-3	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	25.50	24.00
C307-4	Green	Semi Multi	Absent	Absent	Strong	Gynoic	12.00	28.00	24.50
C307-5	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	28.50	24.50
C307-6	Green	Semi Multi	Absent	Absent	Strong	Gynoic	9.00	28.70	25.50
C307-7	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	26.00	24.00
C307-8	Green	Semi Multi	Absent	Absent	Strong	Gynoic	11.00	27.00	23.70
Average							10.88	28.18	25.03
C340-1	Dark Green	Single	Absent	Absent	Very Strong	Gynoic	11.00	30.00	26.00
C340-2	Dark Green	Single	Absent	Absent	Very Strong	Gynoic	11.00	30.00	27.00
C340-3	Dark Green	Single	Absent	Absent	Very Strong	Gynoic	10.00	30.50	25.50
Average							10.67	30.17	26.17
C350-1	Light Green	Single	Absent	Absent	Very Strong	Monoecious	9.00	27.40	25.00
C350-2	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	10.00	27.70	25.00
C350-3	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	9.00	28.90	25.40
C350-4	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	7.00	29.00	25.60
C350-5	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	9.00	22.30	21.00
C350-6	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	10.00	29.20	28.00
C350-7	Light Green	Multi	Absent	Absent	Very Strong	Monoecious	7.00	28.00	27.00
C350-8	Light Green	Semi Multi	Absent	Absent	Very Strong	Monoecious	8.00	28.00	25.00
Average							8.63	27.56	25.25

F_2 Code	Fruit Color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C357-1	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	12.00	32.00	26.00
C357-2	Dark Green	Semi Multi	Absent	Absent	Medium Strong	Gynoic	12.00	34.50	27.60
C357-3	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	10.00	35.00	27.00
C357-4	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	14.00	31.80	27.20
C357-5	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	10.00	29.60	22.00
C357-6	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	10.00	30.00	27.90
C357-7	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	13.00	25.90	22.60
C357-8	Dark Green	Multi	Absent	Absent	Medium Strong	Gynoic	12.00	29.90	26.00
Average							11.63	31.09	25.79

Among the lines with gherkin fruit type, all F_2 plants of line C-355 had dark green fruit color, semi-multi fruit set, warty and spiny fruit, strong plant growth and gynoic flower structure, while F_2 plants of line N-285 had green leaf color, multi fruit set, spiny fruit set, strong plant growth and gynoic flower structure. The average internode length of F_2 plants of line N-285 was (1.5 cm) longer, leaf width (1.5 cm) shorter and leaf length (0.90 cm) longer than line C-355 (Table 6).

Table 6. Fruit color, fruit set, fruit spine, fruit wart, plant vigor, flower structure, internode length, leaf width and length of Gherkin type lines

F_2 Code	Fruit Color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C355-1	Dark Green	Semi Multi	Present	Present	Strong	Gynoic	11.00	24.00	17.90
C355-2	Dark Green	Semi Multi	Present	Present	Strong	Gynoic	15.00	30.20	25.30
C355-3	Dark Green	Semi Multi	Present	Present	Strong	Gynoic	12.00	29.10	23.70
C355-4	Dark Green	Semi Multi	Present	Present	Strong	Gynoic	11.00	35.10	29.20
Average							12.25	29.60	24.03
N285-1	Green	Multi	Present	Present	Very Strong	Gynoic	13.00	28.40	29.40
N285-2	Green	Multi	Present	Present	Very Strong	Gynoic	13.00	26.10	20.70
N285-3	Green	Multi	Present	Present	Very Strong	Gynoic	16.00	32.00	26.80
N285-4	Green	Multi	Present	Present	Very Strong	Gynoic	13.00	25.90	22.80
Average							13.75	28.10	24.93

The fruit color of all F_2 plants belonging to 3 lines with Long European type fruit was found to be dark green. While uniformity was observed in lines C-295 and C-312 in terms of fruit set, single fruit set was observed in 8 F_2 plants of line C-348. While ribless fruit shape and very strong plant growth were observed in F_2 plants of lines C-

395 and C-312, warty and spiny fruit shape and strong plant growth were observed in plants of line C-348. While the plants of line C-312 had monoic flower structure, the plants of the other two lines had gynoic flower structure. The longest internode length and leaf width averages were obtained from the plants of line C-312, while the longest leaf average was obtained from the plants of line C-295 (Table 7).

Table 7. Fruit color, fruit set, fruit spine, fruit wart, plant vigor, flower structure, internode length, leaf width and length of Long European type lines

F_2 Code	Fruit color (Dark Green, Green, Light Green)	Number of fruits per node (Multi, Semi-Multi, Single)	Fruit Spine (Present, Light, Absent)	Fruit wart (Present, Absent)	Plant Growth (Very Strong, Strong, Medium Strong)	Flower Structure (Monoecious, Gynoic)	Internode Length (cm)	Leaf Width (cm)	Leaf Length (cm)
C295-1	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	9.00	28.50	28.30
C295-2	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	10.00	26.00	24.50
C295-3	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	10.00	32.10	28.60
C295-4	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	9.00	34.30	30.10
C295-5	Dark	Single	Absent	Absent	Very Strong	Gynoic	8.00	30.00	28.40
C295-6	Dark	Single	Absent	Absent	Very Strong	Gynoic	9.00	24.50	23.00
C295-7	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	9.00	27.80	26.00
C295-8	Dark	Semi Multi	Absent	Absent	Very Strong	Gynoic	9.00	31.00	27.70
Average							9.13	29.28	27.08
C312-1	Dark	Semi Multi	Absent	Absent	Very Strong	Monoecious	14.00	32.10	28.60
C312-2	Dark	Semi Multi	Absent	Absent	Very Strong	Monoecious	12.00	26.50	24.60
C312-3	Dark	Semi Multi	Absent	Absent	Very Strong	Monoecious	10.00	32.40	29.10
C312-4	Dark	Semi Multi	Absent	Absent	Very Strong	Monoecious	10.00	31.30	16.80
C312-5	Dark	Single	Absent	Absent	Very Strong	Monoecious	12.00	37.10	30.00
C312-6	Dark	Single	Absent	Absent	Very Strong	Monoecious	9.00	36.00	30.50
C312-7	Dark	Single	Absent	Absent	Very Strong	Monoecious	10.00	18.60	18.00
Average							11.00	30.57	25.37
C348-1	Dark	Single	Present	Present	Strong	Gynoic	12.00	23.00	17.00
C348-2	Dark	Single	Present	Present	Strong	Gynoic	11.00	21.00	16.00
C348-3	Dark	Single	Present	Present	Strong	Gynoic	11.00	27.10	16.40
C348-4	Dark	Single	Present	Present	Strong	Gynoic	11.00	25.00	20.40
C348-5	Dark	Single	Present	Present	Strong	Gynoic	10.00	23.80	24.60
C348-6	Dark	Single	Present	Present	Strong	Gynoic	11.00	28.10	23.30
C348-7	Dark	Single	Present	Present	Strong	Gynoic	10.00	24.20	26.40
C348-8	Dark	Single	Present	Present	Strong	Gynoic	10.00	22.00	18.20
Average							10.75	24.28	20.29

Principal component analysis (PCA) was performed to classify F_2 plants based on fruit color, fruit set, fruit shape, plant growth, fruit type, flower structure and fruit color of 109 cucumber plants with different fruit types (Figure 3). According to the analysis, two principal components (68.52% according to PC1 and 14.37% according to PC2) accounted for about 83% of the total variation. F_2 plants with gynoic flower structure, medium strong plant growth and long internodes were mostly located in regions I. and IV. of the graph, while plants with light green fruit color, monoic flower structure, single fruit set and spine fruit shape were mostly located in regions II. and III. of the graph. Plants with high leaf width and length are located in the III. and IV. region of the graph. Plants with multi fruit set and spine fruit shape were mostly located in regions I and IV of the graph. 109 F_2 plants obtained by selfing 16 F_2 lines with different fruit types were located in 4 regions of the graph. The positioning of the plants in different regions shows that there is a wide variation among F_2 plants in terms of the parameters examined.

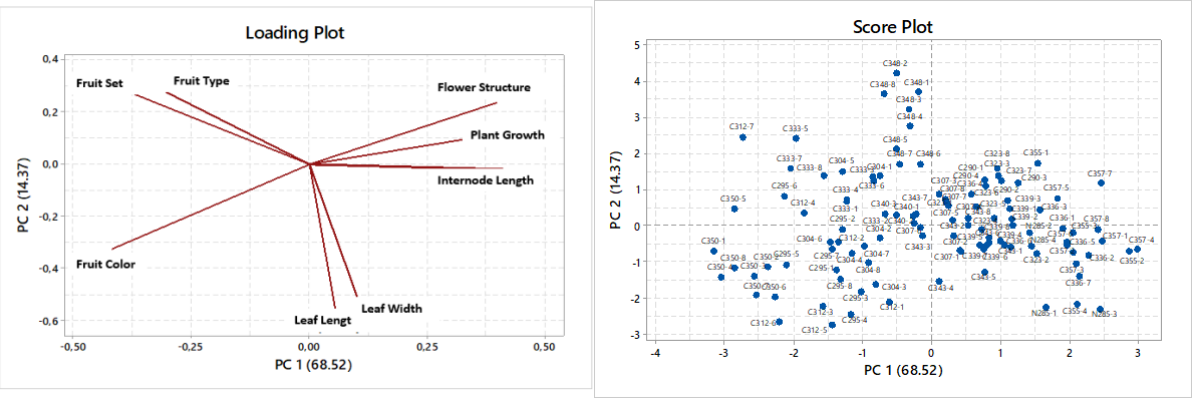

Figure 3. PCA graph of morphological and pomological characteristics of F_2 cucumber lines with different fruit types

DISCUSSION

Many different cucumber varieties with different fruit characteristics such as shape and texture are being studying by breeders to meet consumers demands. For many years, many cucumber varieties with different fruit characteristics have been developed in different parts of the world. İn cucumber breeding programs, the characteristics of the starting material need to be fully characterized. Identification in the gene pool is done by characterization studies. Plant characterization means the identification of the available genetic material. Morphological or agronomic characterization is carried out to transfer inherited traits. The data collected during characterization is considered very useful as it helps to easily distinguish one genetic material from another and also helps to identify genotypes and lines with important traits. This study, morphological and agronomic characterization studies were carried out on 16 hybrid lines at F_2 generation. In terms of fruit type, it was determined that 9 lines of Beith Alpha, 3 lines of Long European, 2 lines of gherkin and 2 lines of Mini (snack) had fruit type. While fruit weight and fruit length of Beith Alpha and Long European type cucumber lines were higher than silor and gherkin type lines, there was not much difference between the types in terms of fruit diameter parameter. Shimomura et al., (2017) reported that cucumber genotypes with different fruit length and weight had no statistical difference in fruit diameter. In parallel with our results, Grumet et al., (2022) reported that parthenocarpic, greenhouse-grown cucumber types with long fruits ranged between 20-40 cm and those with short fruits between 12-15 cm. Kumar et. al., (2013) completed a characterization study on thirty-two cucumber genotypes showed that there was a wide variation among genotypes in terms of average fruit weight, powdery mildew tolerance and aphid damage. Fruit size and shape, especially fruit length, were important fruit characteristics during cucumber domestication and varietal selection (Zhang et al., 2021).Fruit flesh firmness in cucumber is important for the preservation of fruit quality in storage and transportation. Especially since mini (snack) and gherkin type cucumber genotypes are used in pickling and in order to preserve the crispness of the pickle, the fruit flesh firmness should be high. In our study, mini (snack) and gherkin type cucumber lines had the highest fruit flesh firmness. The fruit color of the cucumber lines used in the study varied from light green to dark green. Especially the different fruit color variation in each fruit type will be able to meet the desired fruit color demand for the market in the coming years. Compared to light green skinned fruits, dark skinned fruits have a higher number of chloroplasts and higher levels of chlorophyll a, chlorophyll b and carotenoids. Dark green fruit skins have higher flavonoid and anthocyanin content as well as greater antioxidant activity (Bo et al., 2012; Miao et al., 2019; Jo et al., 2022).

In our study, one of the cucumber lines of long european type cucumber had a spiny fruit surface, while two of them did not have spines on the fruit surface. Both of the gherkin type cucumber lines had spiny fruit surfaces. Fruit surfaces of Beith Alpha and mini (snack) type lines were without warts. Fruit skin characteristics such as spine size and color, fruit warts, dull and uniform color are some of the most important external quality characteristics, along with size and shape, which determine commercial types. All these characteristics are related to the market value of cucumber (Koyama, 1986; Valcárcel et al., 2018). Western fresh market cucumber is generally smooth at harvest, while the western pickling type has a surface with sparse, rounded spines. Northern Chinese cucumbers have large, broad warts covering the fruit surface. Fruits with smoother surfaces are increasingly preferred for commercial production, as the presence of warts and spines can interfere with packing and processing of the fruit (Grumet et al., 2022). Since gherkin-type cucumbers are used in pickling, the high number of surface spines causes the fruit peel to thicken and this contributes to the preservation of pickle quality during the pickle processing process by increasing the hardness of the fruit flesh. In our study, while monoic or gynoic flower structure was observed in each fruit type, having lines with these two flower structures is important both during breeding and for increasing yield and fruit quality, especially gynoic cucumber genotypes form parthenocarpic fruits.

CONCLUSION

As a result of the characterization study, since there is a wide variation among the F_2 plants in terms of the traits examined in each fruit type, the genetic material we have can constitute the starting material for future breeding studies. In addition, the identification of these traits of the lines in the early period, in the F_2 generation and recording these traits will prevent the loss of traits in the later generations and the identification of the genetic factors controlling these traits in future studies will facilitate the breeder's work in terms of less time, labor and financial support in increasing yield and quality, improving transportation, handling and storage characteristics and developing varieties with the quality desired by the consumer.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Authors' individual contributions to the article are equal.

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Greenhouse gas (GHG) emissions in fruit production-I: berries, nuts and citrus

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Abstract

The greenhouse gas emission values obtained from agricultural activities such as tillage, pruning, spraying, fertilizing, harvesting and transporting etc. are not at a negligible level when compared to other sectors. Each practice has an energy input amount. There have been studies on many agricultural products all over the World. Therefore, the carbon dioxide equivalent ($CO_{2\text{-eq}}$) emissions associated with agricultural processes were compared in this study with selected berry, nut and citrus fruits.

As a result; when the greenhouse gas emission equivalent values are examined on a fruit basis, strawberry fruit has 34517.75 kg $CO_{2\text{-eq}}$ per ha amount has the highest input. Wolfberry fruit also follows strawberry fruit with a value of 20718.66 kg$CO_{2\text{-eq}}$ per ha. The minimum greenhouse gas emission equivalent is in tangerine fruit with 399.89 kg $CO_{2\text{-eq}}$ per ha.

Keywords: GHG, Berry, Nuts, Citrus, Fruit

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INTRODUCTION

Energy is an important factor for both nature and social life. Energy production, transformation and consumption are considered important inputs for the environment and sustainable development. Energy production causes significant environmental problems that harm the ecosystem. Energy systems emit various emissions into the environment at different stages from energy production to consumption and disposal. The most important of these is greenhouse gas (GHG) emissions. GHG is a gas in an atmosphere that absorbs and spreads radiation within the thermal infrared range. Keeping these emissions to a minimum is necessary for sustainable development. Energy use, GHG emissions and their potential effects on global climate change are among the current discussions. In this context, increased energy consumption leads to significant environmental issues such as GHG emissions that harm human health.

The natural environment is affected by all human activities. Fossil fuel consumption, which has continued to increase since the beginning of the industrial revolution and has reached very high levels, is the main reason for the emergence of energy environmental problems. Environmental effects caused by energy production can be listed as acid pollutants, global warming, human health and safety problems, particles, heavy metals, disaster probability, waste problems, bad images, noise, light pollution, radiation pollution and land use.

The total greenhouse gas emissions was 219.5 million tonnes $CO_{2\text{-eq}}$ in 1990 and increased to 564.4 million tonnes $CO_{2\text{-eq}}$ in 2021. The share of agriculture is equal to 21.00% in 1990 and 12.77% in 2021 (TUIK, 2024).

In this study, published papers till 2024 on energy analysis in berry, nuts and citrus production were evaluated in terms of GHG emissions. The total energy input and total $CO_{2\text{-eq}}$ values were given and the percentage of input parameters on $CO_{2\text{-eq}}$ were also given as graphs.

MATERIALS AND METHODS

Greenhouse gas emissions are calculated by using the 2006 Intergovernmental Panel on Climate Change (IPCC) Guidelines. The greenhouse gas emissions statistics press release includes carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases (F-gases) which are direct GHGs originating from energy, industrial processes and product use, agriculture and waste sectors. İndirect GHG emissions from nitrogen oxides (NO_x), non-methane volatile organic compounds (NMVOC), carbon monoxide (CO) and sulphur dioxide (SO_2) are not included. Emissions and removals from land use, land use change and forestry are not included in the press release (TUIK, 2024).

Agricultural production necessitates the use of a variety of input materials such as fertilizers, biocides, seeds, and energy carriers such as natural gas and diesel fuel. The production, formulation, storage, and distribution of agricultural inputs result in the use of energy sources that trigger the combustion of fossil fuels and the release of carbon dioxide (CO_2) and other greenhouse gases into the atmosphere (Lal, 2004).

This study compares GHG emission values in different norms using prior research and statistical data in berry, nut and citrus production. These fruits are berries as grape, pomegranate, strawberry, wolfberry, nuts as almond, pistachio, walnut, citrus as orange, lemon, mandarin and grapefruit.

The following equation is used to define the GHG emissions;

$$GHG_{ha} = \sum_{i=1}^{n} R(i) \times EF(i)$$

GHG_{ha}: GHG emission $\left(kg\ CO_{2-eq}/ha\right)$,
$R(i)$: Amount of i input $\left(unit_{input}/ha\right)$,
$EF(i)$: GHG emission equivalent of i input $\left(kg\ CO_{2-eq}\ per\ input\right)$.

In this study, only published papers were evaluated according to the each fruit. So, the place and country were given in the graphs and text. In some fruits there are limited information in the literatures.

Table 1. Greenhouse gas (GHG) emissions coefficients in agriculture production

Input Parameters	Unit	GHG Coefficient (kg $CO_{2\text{-eq}}$per unit)	References
Human	h	0.700	Eren et al., 2019a
Animal	h	0.043	Gokdogan et al., 2022
Diesel	L	2.760	Eren et al., 2019b
Gasoline	L	2.350	Annoymous, 2024
Propane	L	1.525	Annoymous, 2024
Farm Machinery	MJ	0.071	Agizan et al., 2024
Pesticides	kg	5.210	Alizadeh and Taromi, 2014
Herbicides	kg	6.300	Khoshnevisan et al., 2014
Insecticides	kg	5.100	Moe et al., 2024
Fungucides	kg	3.900	Taki et al., 2013
Organic Chemicals	kg	5.100	Agizan et al., 2024
Acaroids	kg	5.100	Ozalp et al., 2018
Farmyard Manure	ton	0.005	Baran et al., 2023
Chemical Fertilizer	kg	4.550	Ekinci et al., 2020
Nitrogen	kg	1.300	Pishgar-Komleh et al., 2012
Phosphate	kg	0.200	Seydosoglu et al., 2023
Potassium	kg	0.200	Kazami and Zardari, 2018
Sulphur	kg	0.370	Sari and Gokdogan, 2024
Lime	kg	0.390	Ekinci et al., 2020
Ferrum	kg	4.550	Ekinci et al., 2020
Microelements	kg	4.550	Ekinci et al., 2020
Organic Fertilizer	kg	0.129	Agizan et al., 2024
Electricity	kWh	0.608	Candemir et al., 2024
Water	m^3	0.170	Kulekci and Sari, 2020
Transportation	ton km	0.150	Gokdogan et al., 2024
Plastic	kg	3.120	Annoymous, 2024

RESULTS AND DISCUSSION

BERRIES

Grape

Figure 1 shows the total energy input and $CO_{2\text{-eq}}$ values in grape production in the World. The highest GHG amount was 6257.4 kg $CO_{2\text{-eq}}$ per ha in the West Azerbaijan (Iran) (Mardani and Taghavifer, 2016). When the total energy input values were evaluated, it reached to 82193.2 MJ/ha in East Azerbaijan (Iran) (Sattari-Yuzbashkandi et al., 2014). It was minimum in Thrace Region (Turkey) (Akdemir, 2022) with 12144.6 MJ/ha.

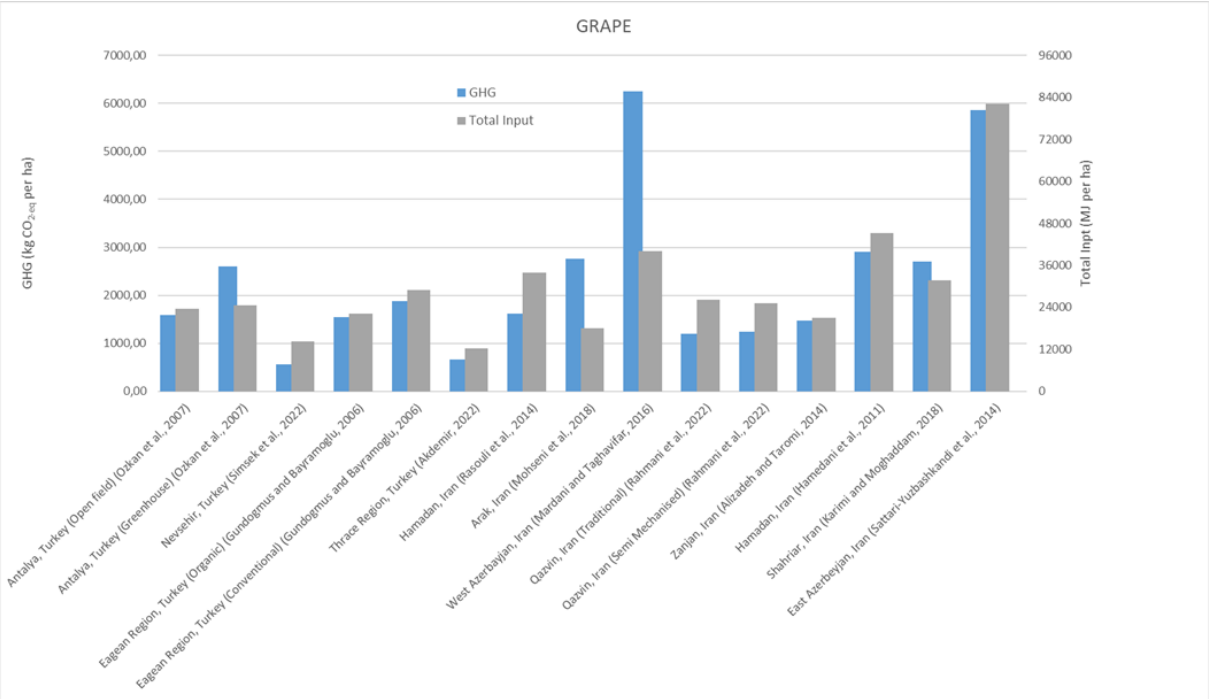

Figure 1. $CO_{2\text{-eq}}$ and total energy input values in grape production in the Iran and Turkey.

Figure 2 shows input rates in grape production in the World and Turkey. In grape production, the input rate in human labor was used the most in Antalya (Turkey) (Ozkan et al., 2007) with 57.45%. In Nevsehir (Turkey) (Simsek et al., 2022), the input rates in farm machinery and diesel were used the most with 26.92% and 37.73%, respectively, as seen in Figure2. The highest pesticide use is in Zanjan (Iran) (Alizadeh and Taromi, 2014) with a rate of 29.48%. Although the use of herbicides and insecticides is low, the rate of fungicide is 12.64% in the Eagean Region (Turkey) (Gundogmus and Bayramoglu, 2006). Nitrogen was used the most in Qazvin (Iran) (Rahmani et al., 2022) with a rate of 20.40% among chemical fertilizers. In the Thrace Region (Turkey) (Akdemir, 2022), the input in sulphur is the highest with a rate of 13.88%, as seen in Figure 2. The electricity input parameter is in Hamadan (Iran) (Rasouli et al., 2014) with a rate of 25.62%. Among the parameters used in grape production, the highest input rate is in the West Azerbaijan (Iran) (Mardani and Taghavifer, 2016) region with 63.90%.

Pomegranate

In pomegranate production, the maximum GHG emission value is 4307.4 kg $CO_{2\text{-eq}}$ per ha in Mazandaran (Iran) (Nouri-Khjebelagh et al., 2023) as seen in Figure 3. Its' minimum value was 832.3 kg $CO_{2\text{-eq}}$ per ha in Mazandaran (Iran) (Troujeni et al., 2018) in another study. The total energy input was ranged between 11195.1 in Mazandaran (Iran) (Troujeni et al., 2018) and 54934.6 MJ/ha in Antalya (Turkey) (2.1-4.0 ha) (Ozalp et al., 2018).

Figure 4. shows the input rates in pomegranate production in the World. Berry fruits are mostly collected by hand. Therefore, the human factor is among the important parameters. In pomegranate production, the input rate of human labor is 41.22% in Antalya (Turkey) (Akcaoz et al., 2009) as seen in Figure 4. The input in diesel was used the most in Mazandaran (Iran) (Troujeni et al., 2018) with a rate of 30.20%. Figure 4 shows that, the input rate of farm machinery is used the most in Antalya (Turkey) (Ozalp et al., 2018) with 13.91%. There is no pesticide use in pomegranate production in Turkey. However, the highest pesticide use is in Fars (Iran) (34.39%) (Housyar et al., 2017). The use of herbicides, insecticides and fungicides in pomegranate fruit is low. The input rates of these chemicals in Antalya (Turkey) are 3.43%, 5.81% and 9.92%, respectively. The use of farmyard manure in pomegranate production is low with a rate of 0.2% in Antalya (Turkey) (Ozalp et al., 2018). The chemical fertilizers used in pomegranate production are nitrogen, phosphate and potassium. Among these fertilizers, nitrogen was used the most in Antalya (Turkey) (Ozalp et al., 2018) with a rate of 21.34%. Electricity use in

Antalya (Turkey) (Ozalp et al., 2018) is 29.08%. It is seen in Figure that, water is used the most in Mazandaran (Iran) (Nouri-Khjebelagh et al., 2023) with a rate of 63.15%.

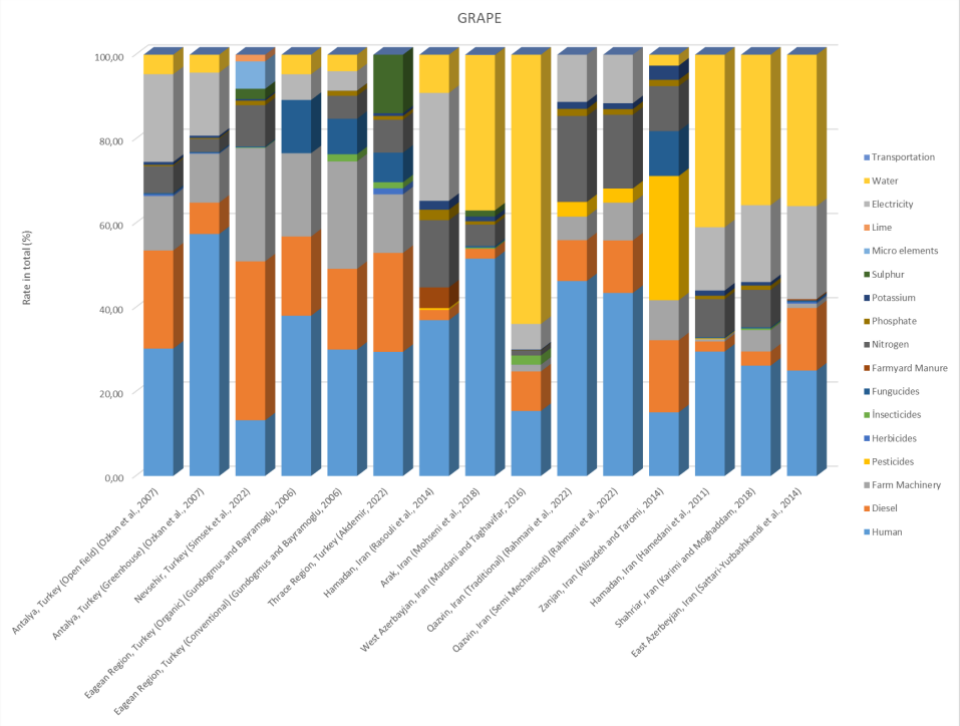

Figure 2. Percentage of input parameters in grape production in the Iran and Turkey

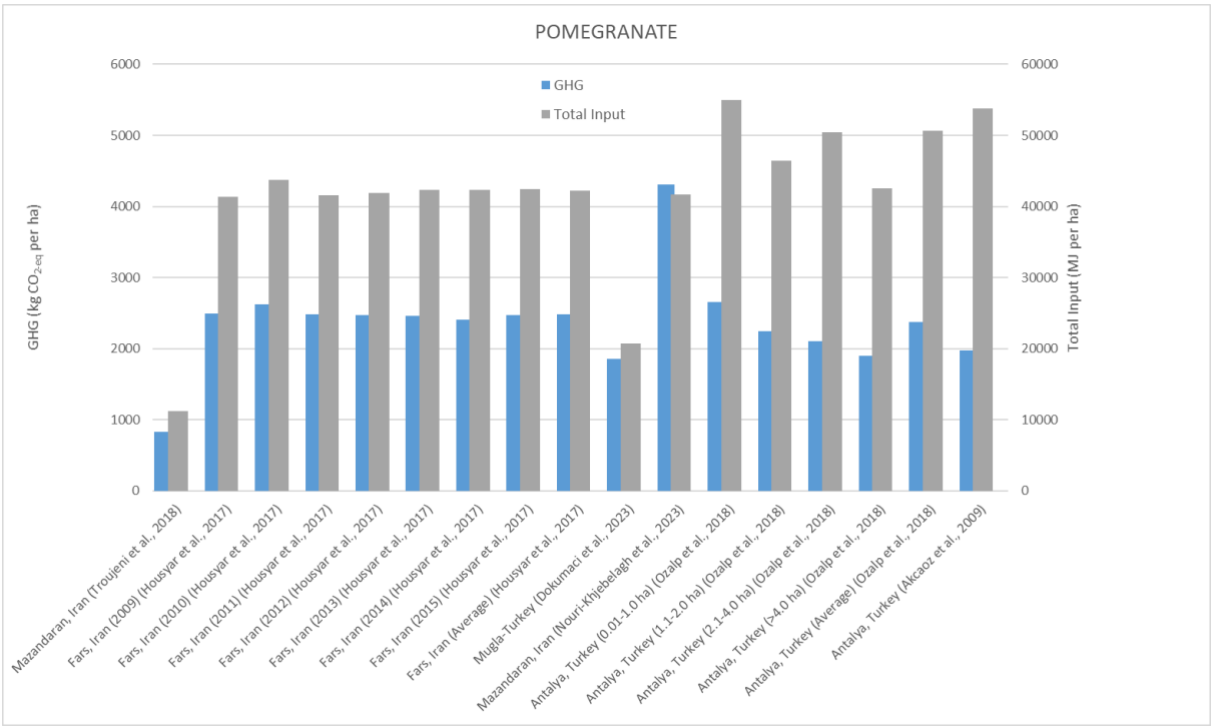

Figure 3. $CO_{2\text{-eq}}$ and total energy input values in pomegranate production in the Iran and Turkey

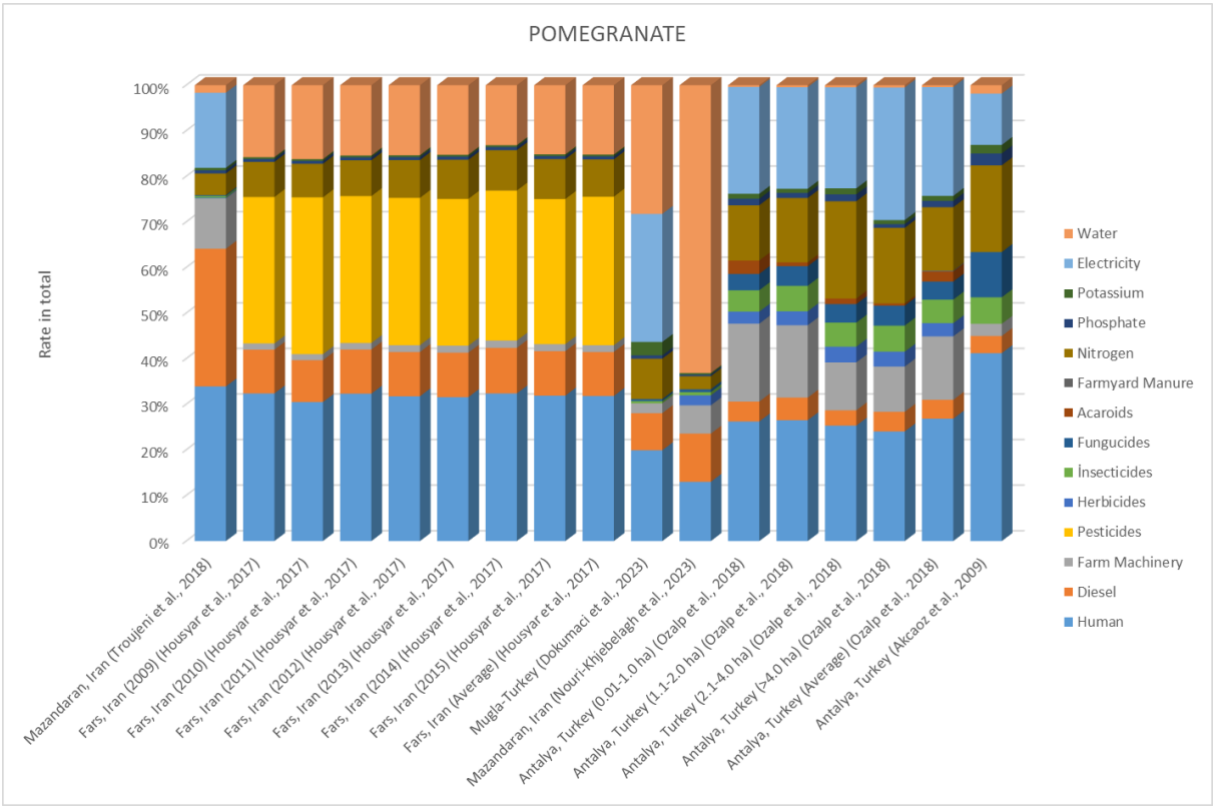

Figure 4. Percentage of input parameters in pomegranate production in the Iran and Turkey

Strawberry

Figure 5 shows the $CO_{2\text{-eq}}$ values in strawberry production in the World. The max GHG emission value was calculated as 34517.76 kg $CO_{2\text{-eq}}$ per ha in Guilan (Iran) (Greenhouse) (Khoshnevisan et al., 2013). This value was 9145.5 kg .$CO_{2\text{-eq}}$ per ha in Nevsehir (Turkey) (Organic) (Baran et al., 2017). The total energy input value ranged between 35092.4 (open field) and 1356932.8 (greenhouse) MJ/ha in Guilan (Iran) (Khoshnevisan et al., 2013).

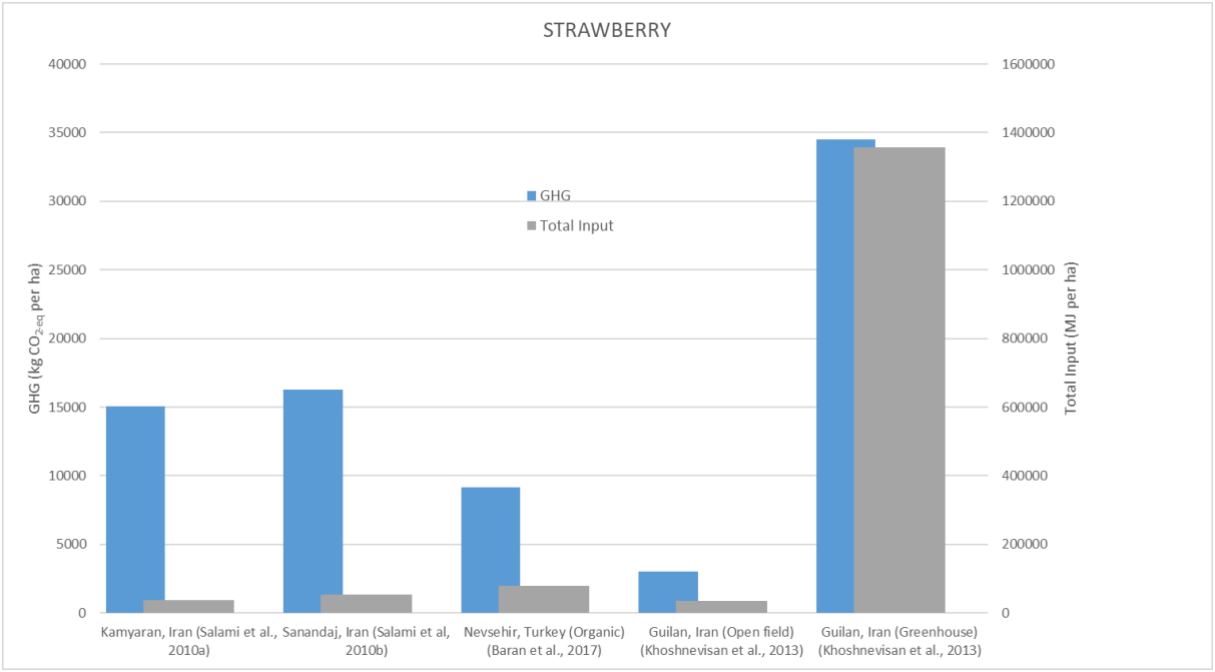

Figure 5. $CO_{2\text{-eq}}$ and total energy input values in strawberry production in the Iran and Turkey

Figure 6. shows the percentage of input parameters in strawberry production in the World. The human factor is of great importance in strawberry production. Therefore, when looking at Figure 6, it is seen that the input rate of human labor is 68.39% in the Guilan (Iran) (Open field) (Khoshnevisan et al., 2013). The use of farm machinery in strawberry production is at low levels. The rate of diesel in total GHG is also low in Guilan (Iran) (Khoshnevisan et al., 2013) with 2.60%. Pesticide use has high rates in Kamyaran and Samandaj (Iran) (Salami et al., 2010a,b). It is used the most in Samandaj (Iran) (Salami et al., 2010b) with a rate of 63.11%. Organic chemicals and fertilizers are used only in Nevsehir (Turkey) (Baran et al., 2017). The rates are 1.25% and 2.82%, respectively. The highest use of chemical fertilizers is in Guilan (Iran) (Khoshnevisan et al., 2013). Among these fertilizers, nitrogen is used the most with a rate of 9.37%, as seen in Figure 6. The GHG rate in the electricity is 54.93% in Guilan (Iran) (Khoshnevisan et al., 2013), where strawberry is grown in greenhouses. The water input rate in strawberry produced in Kamyaran (Iran) (Salami et al., 2010a) is 35.51%. Plastic use in strawberry production is only in Nevsehir (Turkey) (Baran et al., 2017) with a rate of 13.65%.

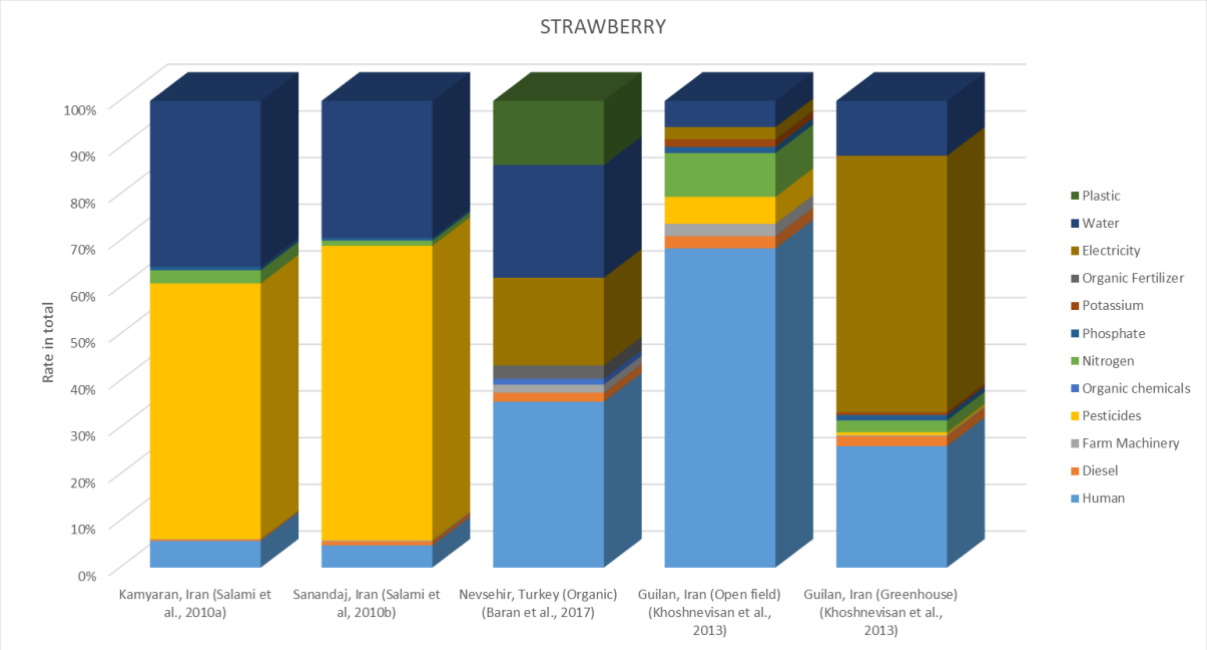

Figure 6. Percentage of input parameters in strawberry production in the Iran and Turkey

Wolfberry

Figure 7 shows the $CO_{2\text{-eq}}$ values in wolfberry production in the World. This value changed between 1343.5 (Aksaray, Turkey) (Oguz et al., 2019) and 20718.7 kg $CO_{2\text{-eq}}$ per ha (Gansu, China) (Wang et al., 2019). The total energy input is 7753.8 MJ/ha in Aksaray (Turkey) (Oguz et al., 2019) and about 40 times higher in Gansu (China) (Wang et al., 2019).

Figure 8 shows the input rates in wolfberry production in the World. Wolfberry production is carried out in Gansu (China) (Wang et al., 2019) and Aksaray (Turkey) (Oguz et al., 2019). It is seen in Figure 8 that, the GHG input in human labor is in Aksaray (Turkey) (Oguz et al., 2019) with a rate of 80.92%. and 28.8% in Gansu (China) (Wang et al., 2019). Diesel and farm machinery parameters in wolfberry production are used more in Aksaray (Turkey) (Oguz et al., 2019) with 6.18% and 6.05%, respectively. The use of organic chemicals is only available in Aksaray (Turkey) (Oguz et al., 2019) with a rate of 1.42%. Chemical fertilizers, pesticides and herbicides were not used in wolfberry production in Aksaray (Turkey) (Oguz et al., 2019). The input rate of herbicides is 0.27% in Gansu (China) (Wang et al., 2019) as seen in Figure 8. Pesticide use was also obtained in Gansu (China) (Wang et al., 2019) as 1.58%. Nitrogen, phosphate and potassium fertilizers were used in Gansu (China) (Wang et al., 2019) and the highest rate was 3.43% in nitrogen fertilizer. Organic fertilizer was used only in Aksaray (Turkey) (Oguz et al., 2019) with a rate of 0.90%. The GHG input in electricity was used only in Gansu (China) (Wang et al., 2019) with a rate of 51.77%. The highest input in water is 10.34% in Gansu (China) (Wang et al., 2019) as seen in Figure 8. The high $CO_{2\text{-eq}}$ value is due to the excessive use of electricity and water inputs in wolfberry production in Gansu (China) (Wang et al., 2019).

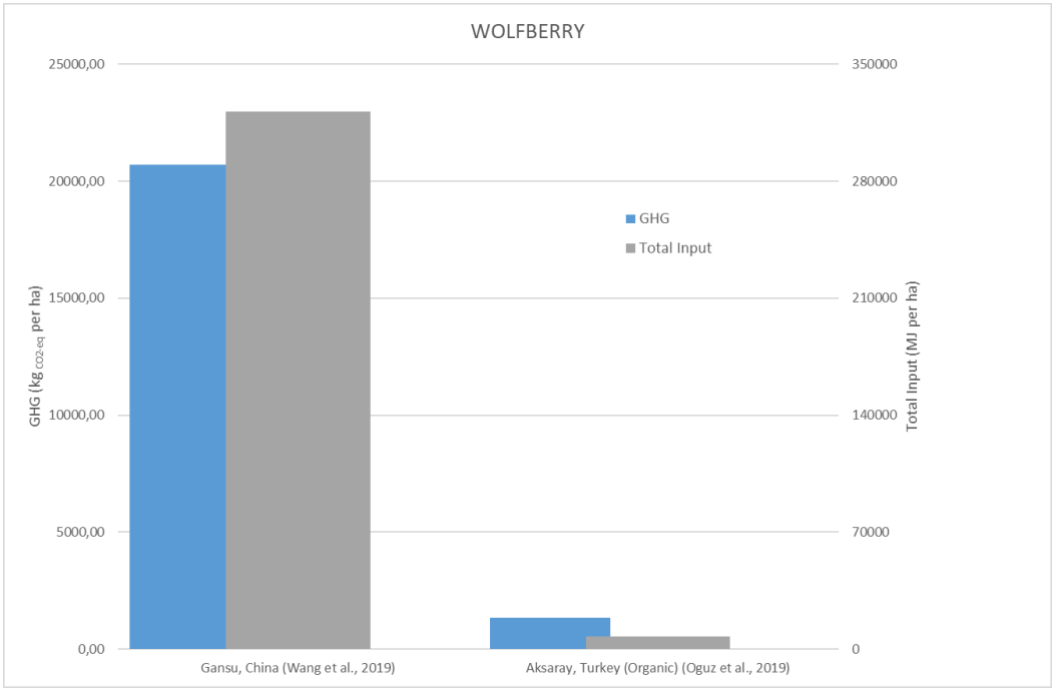

Figure 7. $CO_{2\text{-eq}}$ and total energy input values in wolfberry production in the China and Turkey

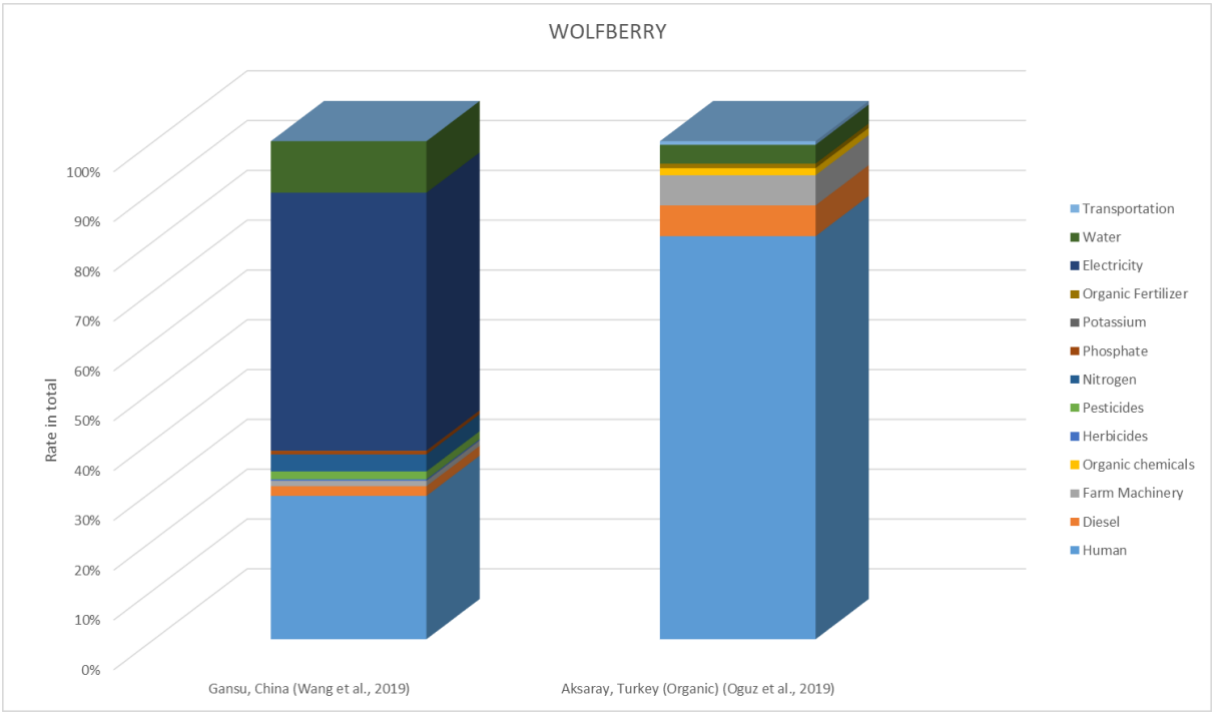

Figure 8. Percentage of input parameters in wolfberry production in the China and Turkey.

NUTS

Almond

Figure 9 shows the $CO_{2\text{-eq}}$ and total energy input values in almond production in the World. The highest GHG emission value was calculated between 2231.7 in Shahrekord (Iran) (Beni et al., 2023) and 6778.0 kg $CO_{2\text{-eq}}$ per ha in the Central Valleys of California (USA) (Pimentel, 1980). It is seen in Figure 9 that, the total energy input value is the highest in the Central Valleys of California (USA) (Pimentel, 1980) with 88491.6 MJ/ha, while it is 19670.4 MJ/ha in Adiyaman (Turkey) (Yilmaz and Beyan, 2023).

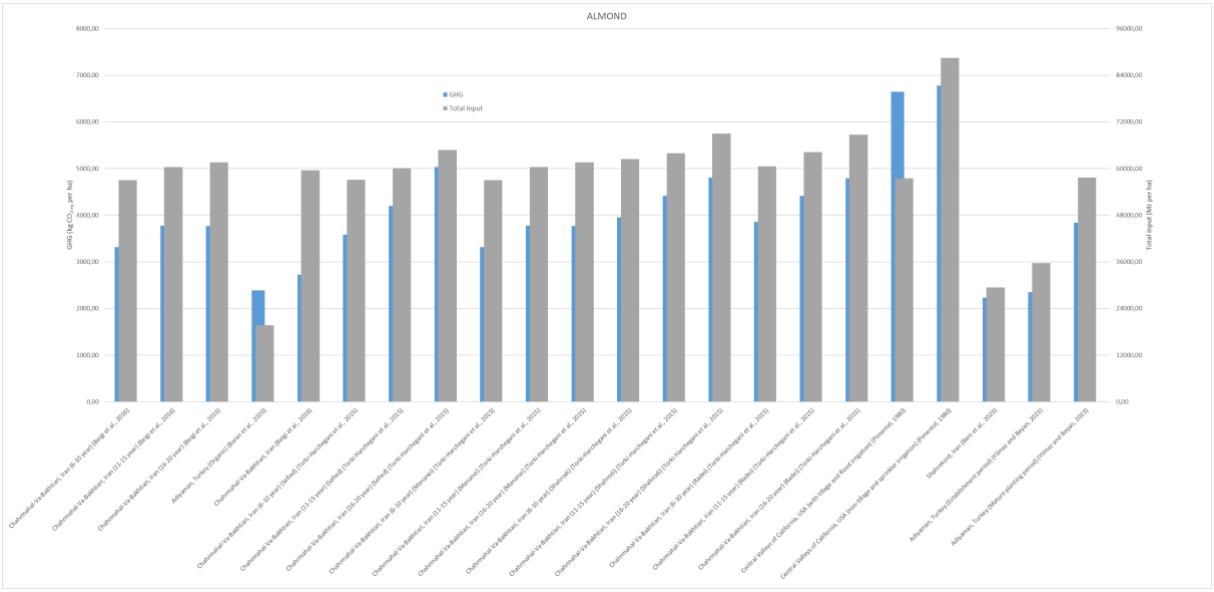

Figure 9. $CO_{2\text{-eq}}$ and total energy input values in almond production in the Iran, USA and Turkey

Figure 10 shows the rates of different input parameters in almond production in the World. It is seen in Figure that, the highest input rate in human labor is in Adiyaman (Turkey) (Yilmaz and Beyan, 2023) with 57.07%. In almond fruit production, fuel energy input parameter was entered in two separate forms as diesel and gasoline. Diesel fuel was used the most in Adiyaman (Turkey) (Yilmaz and Beyan, 2023) with a 15.01% rate. Gasoline was used the most in Central Valleys of California (USA) (Pimentel, 1980) with a 5.38% rate. In almond production, 19.84% was used the most in farm machinery in Shahrekord (Iran) (Beni et al., 2023). Pesticides have the highest usage rate in Shahrekord (Iran) (Beni et al., 2023) and the percentage value is 3.59%. The use of herbicides, insecticides and fungicides in almond production is low. It is seen in Figure 10 that, the rate values of herbicides with 0.43% and fungicides with 0.66% were carried out in Central Valleys of California (USA) (Pimentel, 1980) and insecticides with 5.98% in Chahrmahal-va-Bakhtiari (Iran) (Torki-Harchegani et al., 2015). Lime was used only in Adiyaman (Turkey) (0.07%) (Yilmaz and Beyan, 2023). No farmyard manure was used in almond production. The use of chemical fertilizers in almond production is low. The highest input was nitrogen (8.27%) used in Shahrekord (Iran) (Beni et al., 2023). Sulphur was used only in Shahrekord (Iran) (Beni et al., 2023) with a rate of 0.07%. Propane was calculated only in Central Valleys of California (USA) (Pimentel, 1980) with a rate of 0.83%. Organic chemicals were used only in Adiyaman (Turkey) (Yilmaz and Beyan, 2023) where organic almond is produced with a rate of 0.35%. The highest input of electricity was used in Chahrmahal-va-Bakhtiari (Iran) (Torki-Harchegani et al., 2015) with a rate of 62.31%. The highest rate among the energy parameters in almonds (76.96%) was calculated in the Central Valleys of California (USA) (Pimentel, 1980) for water. Transportation was used the most in the Central Valleys of California (USA) (Pimentel, 1980) with a rate of 7.83%.

Pistachio

Figure 11 shows the $CO_{2\text{-eq}}$ and total energy input values in pistachio production in the World. The GHG emission value is minimum in Southeastern Anatolia (Turkey) (Saglam et al., 2012) with 571.0 and maximum with 3955.75 kg $CO_{2\text{-eq}}$ per ha in Markazi (Iran) (Afshar et al., 2013). Total energy input value is also low in Southeastern Anatolia Region (Turkey) (Saglam et al., 2012) and high in Markazi (Iran) (Afshar et al., 2013) as 12044.0 and 54305.40 MJ/ha, respectively.

Figure 12 shows the energy input rates in pistachio fruit production in the World. The human factor is used intensively in the production of pistachio. Figure 12 shows that, pistachios are used human labor the most in Gaziantep (Turkey) (Kulekci and Aksoy, 2011) with 49.62%. The production carried out using animal power only in Adiyaman (Turkey) (0.36%) (Gokdogan et al., 2022). The percentage value in diesel was used the most in the Southeastern Anatolia (Turkey) (Saglam et al., 2012) with a rate of 33.25%. The rate of farm machinery was realized in Adiyaman (Turkey) (Gokdogan et al., 2022) with a rate of 20.11%. Pesticide is used in pistachio in Adiyaman and Southeastern Anatolia (Turkey) region and the highest input rate is in Southeastern Anatolia (Turkey) (Saglam et al., 2012) with 2.28%. Herbicide, insecticide and fungicide are not used in Adiyaman and Southeastern Anatolia (Turkey). The highest input value is used in Gaziantep (Turkey) (0.1-10 ha) (Kulekci and Aksoy, 2011) with 13.80%. As for herbicide and insecticide, it is seen in Markazi (Iran) (Afshar et al., 2013) with 0.84% and Gaziantep (Turkey) (0.1-10 ha) (Kulekci and Aksoy, 2011) with 0.62%, respectively. Farmyard manure was not used in pistachio production. In pistachio production, nitrogen and phosphate fertilizers are used the most in the Southeastern Anatolia (Turkey) (Saglam et al., 2012) with 25.50% and 3.50%, respectively. Potassium

fertilizer was used the most in Gaziantep (Turkey) (0.1-10 ha) (Kulekci and Aksoy, 2011) with 0.57%. Sulphur and microelements are used only in Adiyaman (Turkey) (Gokdogan et al., 2022) with a very low level (1.24% and 0.28%). Electricity and water input values are used the most in Markazi (Iran) (Afshar et al., 2013) with a rate of 27.73% and 35.94%, respectively.

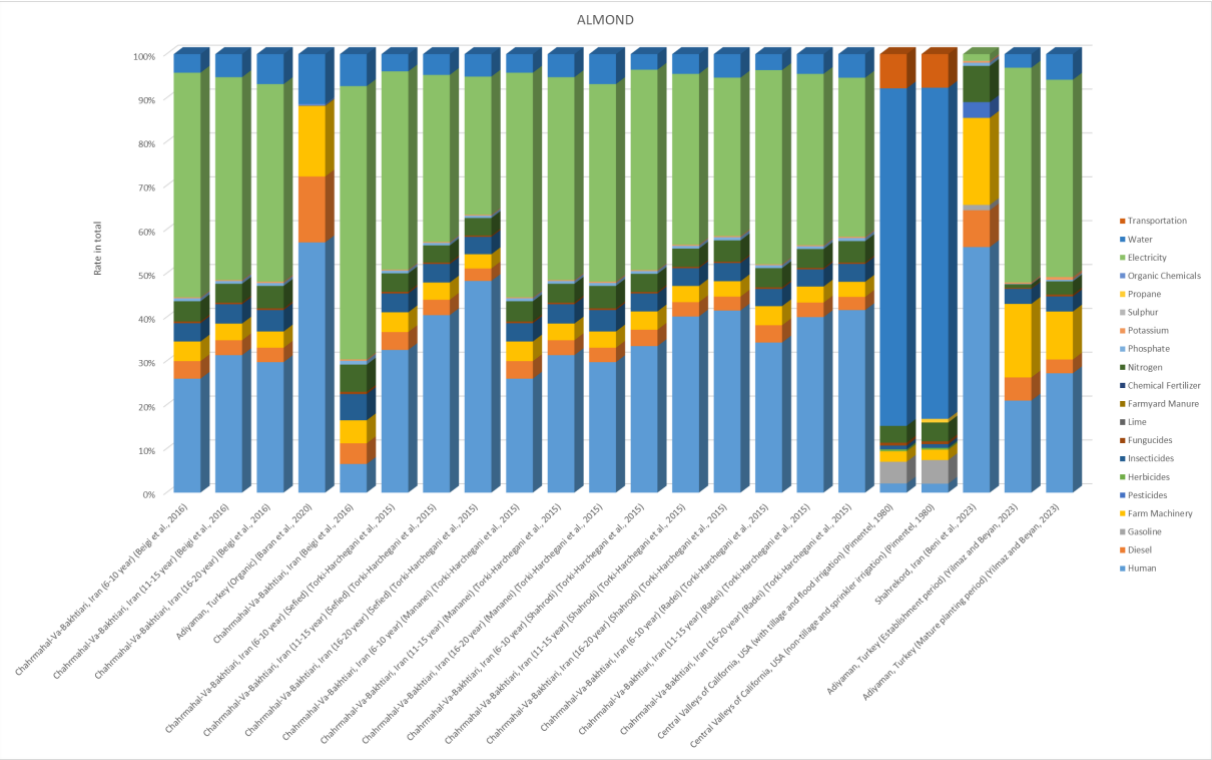

Figure 10. Percentage of input parameters in almond production in the Iran, USA and Turkey

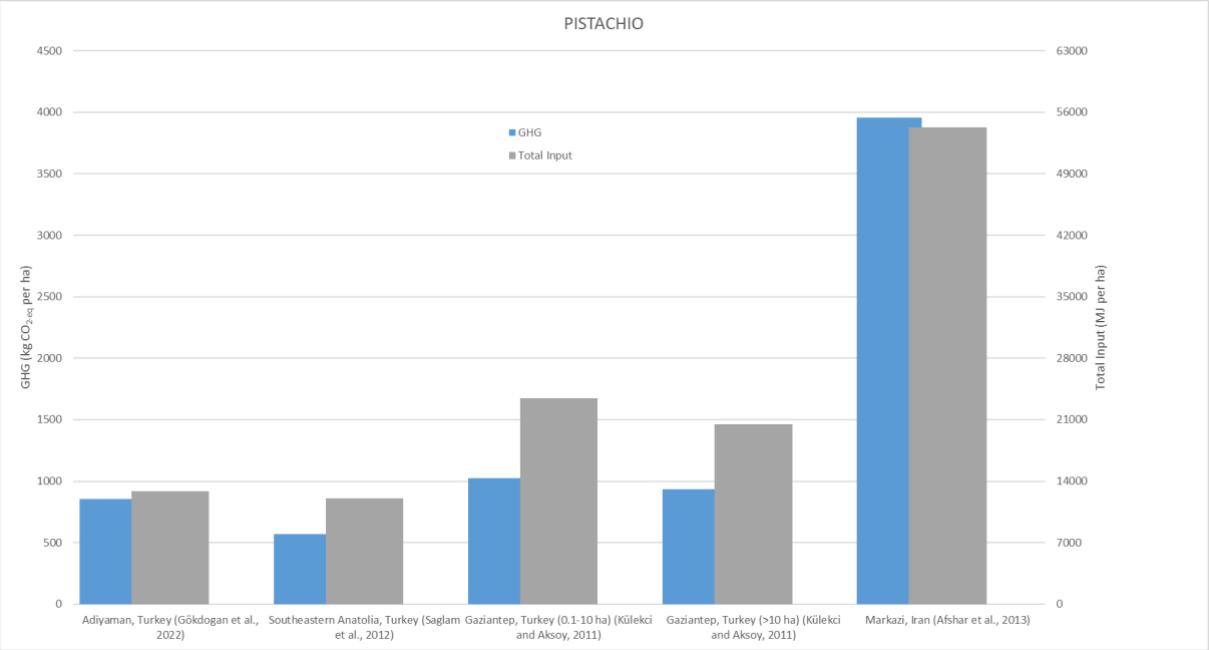

Figure 11. $CO_{2\text{-eq}}$ and total energy input values in pistachio production in the Iran and Turkey

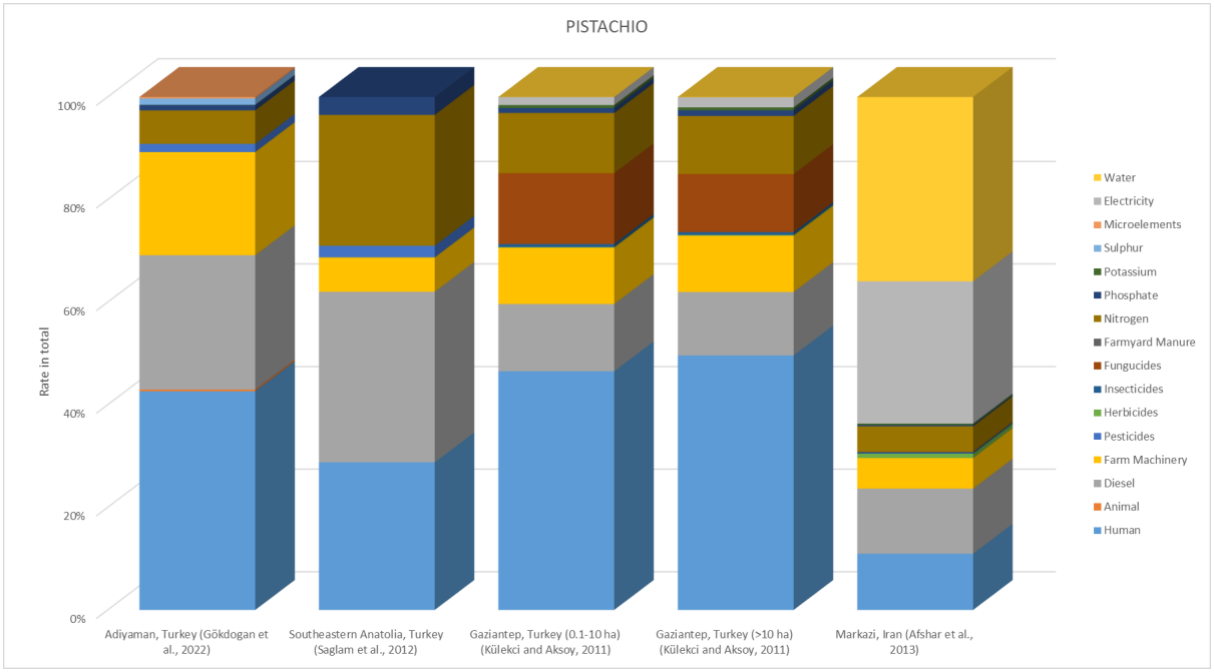

Figure 12. Percentage of input parameters in pistachio production in the Iran and Turkey

Walnut

Greenhouse gas emission in walnut production is seen to be the highest in California (USA) (Pimentel, 1980) with 7099.5 kg $CO_{2\text{-eq}}$ per ha in Figure 13. It is low in Istanbul (Turkey) (Unakitan and Inan, 2020) with a value of 522.1 kg $CO_{2\text{-eq}}$ per ha. The total energy input in walnut production was calculated as 103201.00 MJ/ha. The high rates of input parameters in GHG ensured that, the total energy input value was also high at the same rate. It is minimum with 10096.4 MJ/ha in California (USA) (Pimentel, 1980).

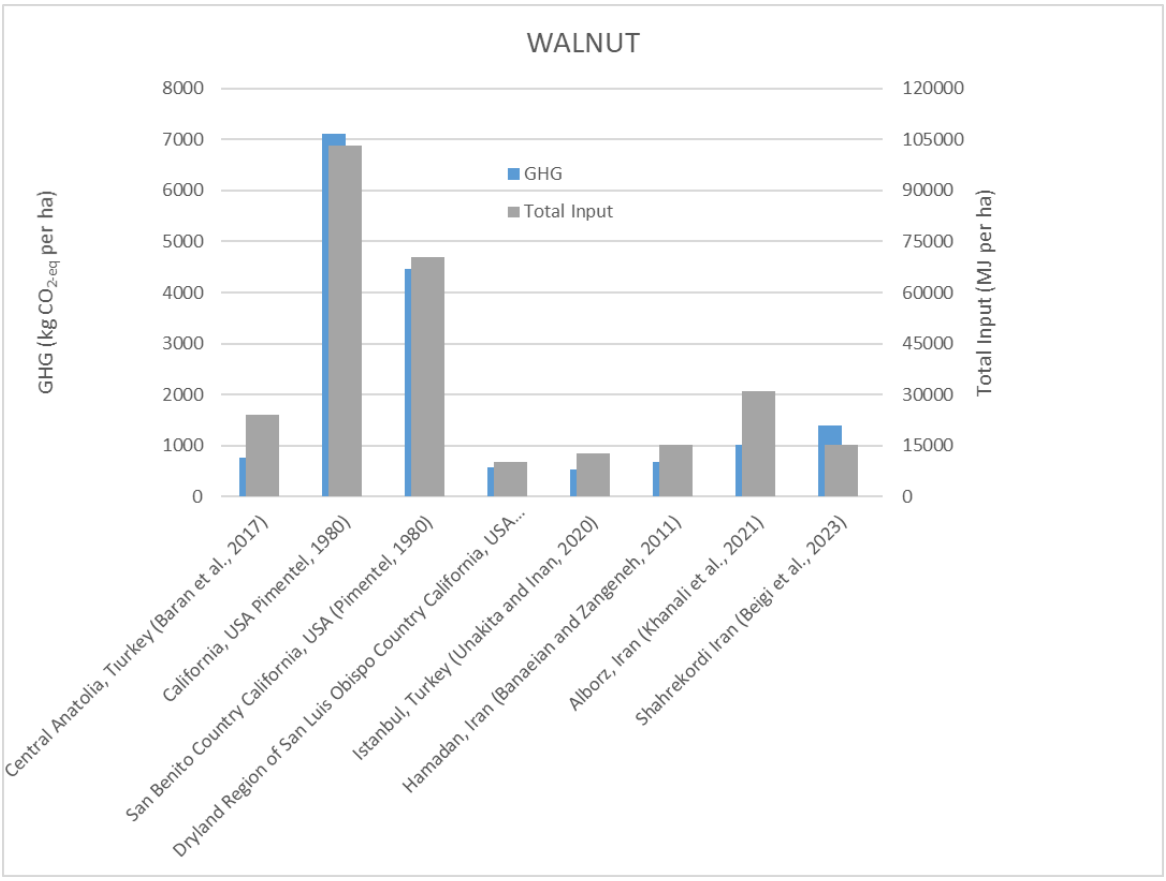

Figure 13. $CO_{2\text{-eq}}$ and total energy input values in walnut production in the Iran, USA and Turkey

Human labor use in walnut production is high and is at the highest level in Shahrekordi (Iran) (Beigi et al., 2023) with a rate of 56.53% as seen in Figure 14. Rate of input parameters in fuel and farm machinery are at their highest rates in the Dryland Region of San Luis Obispo Country California (USA) (Pimentel, 1980). The percentage values in these input parameters are 72.11% and 17.65%, respectively. The pesticide was used in the Central Anatolia Region (Turkey) (Baran et al., 2017), Alborz (Iran) (Khanali et al., 2021), and Shahrekordi (Iran) (Beigi et al., 2023), as seen in Figure 14. The highest input rate was 4.74% in Shahrekordi (Iran) (Beigi et al., 2023). Figure shows that, herbicides, insecticides and fungicides are used in walnut production and the highest rate is 5.79% in Hamadan (Iran) (Banaeian and Zangeneh, 2011). Farmyard manure is used in walnut production to a very small extent in the Central Anatolia Region (Turkey) (Baran et al., 2017) and Hamadan (Iran) (Banaeian and Zangeneh, 2011). Farmyard manure contributed 0.10% in the Central Anatolia Region (Turkey) (Baran et al., 2017) and 0.01% in Hamadan (Iran) (Banaeian and Zangeneh, 2011).

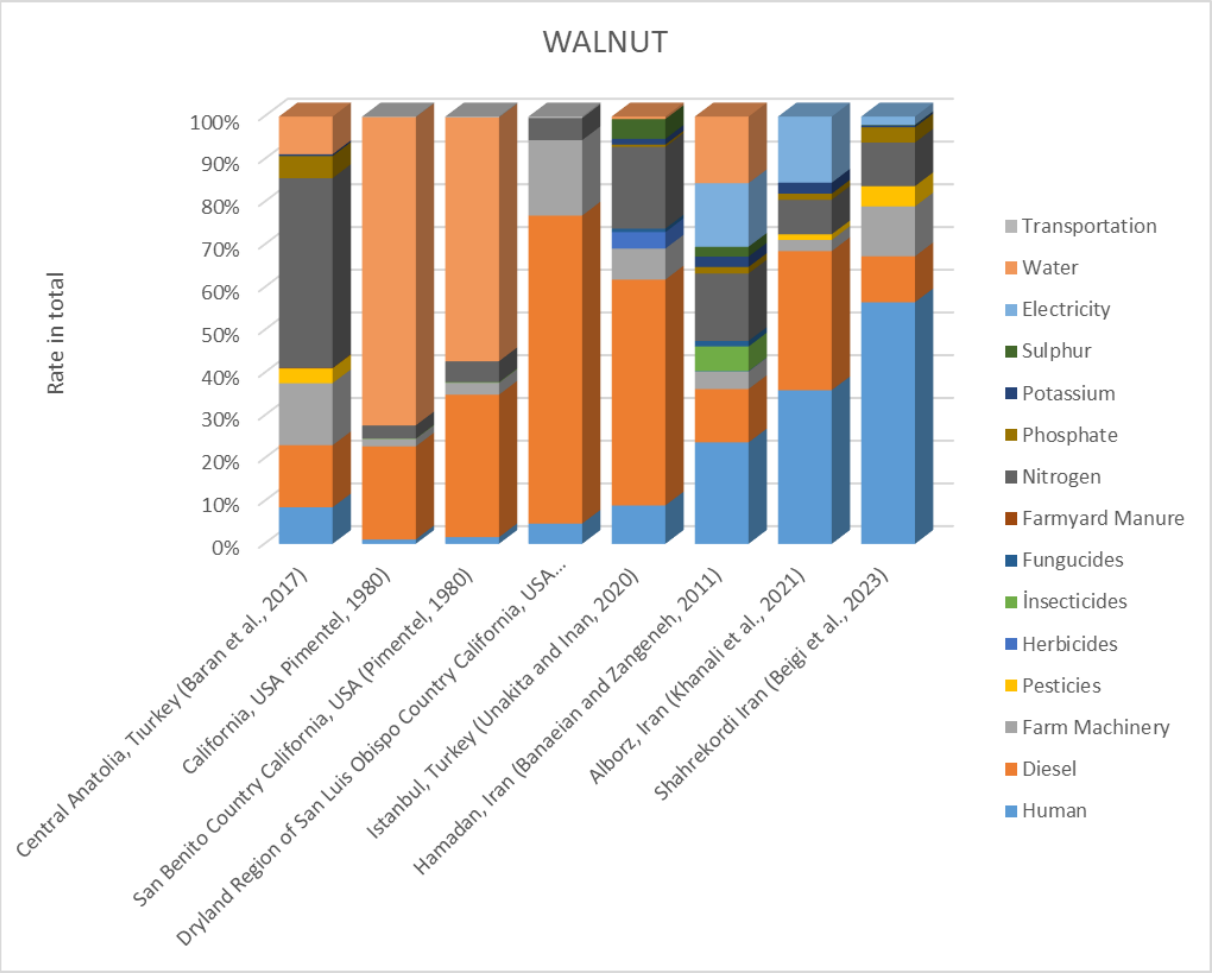

Figure 14. Percentage of input parameters in walnut production in the Iran, USA and Turkey

Nitrogen and phosphate fertilizers are used at the highest values in the Central Anatolia Region (Turkey) (Baran et al., 2017). The percentage values of these fertilizers are calculated as 44.43% and 5.16%, respectively. Potassium fertilizer is used in Alborz (Iran) (Khanali et al., 2021) with a rate of 2.57%, as seen in Figure 14. There is sulphur use in walnut production and it is the highest in İstanbul (Turkey) (Unakitan and Inan, 2020) with a rate of 4.63%. It is seen in Figure 14 that, the input rate in electricity is the highest in Hamadan (Iran) (Banaeian and Zangeneh, 2011) with a rate of 14.88%. The water use in walnut production is 72.15% in California (USA) (Pimentel, 1980). The input rate of transportation is the highest in the Dryland Region of San Luis Obispo Country California (USA) (Pimentel, 1980) with a rate of 0.35%.

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Orange

GHG emission values in orange production in the World are shown in Figure 15. GHG emissions are highest in Kayseri (Turkey), where conventional and organic orange production is carried out. As a result of the high use of chemical fertilizers in this region, GHG emissions are high as 14183.87 in conventional and 10403.35 kg $CO_{2\text{-eq}}$ per ha in organic production. The lowest value is obtained in Mazandaran (Iran) with 1058.3 $CO_{2\text{-eq}}$ per ha.

The total energy input ranged between 23723.8 (North Iran) (<2 ha) and 110361.3 MJ/ha (Mazandaran, Iran) (7th year).

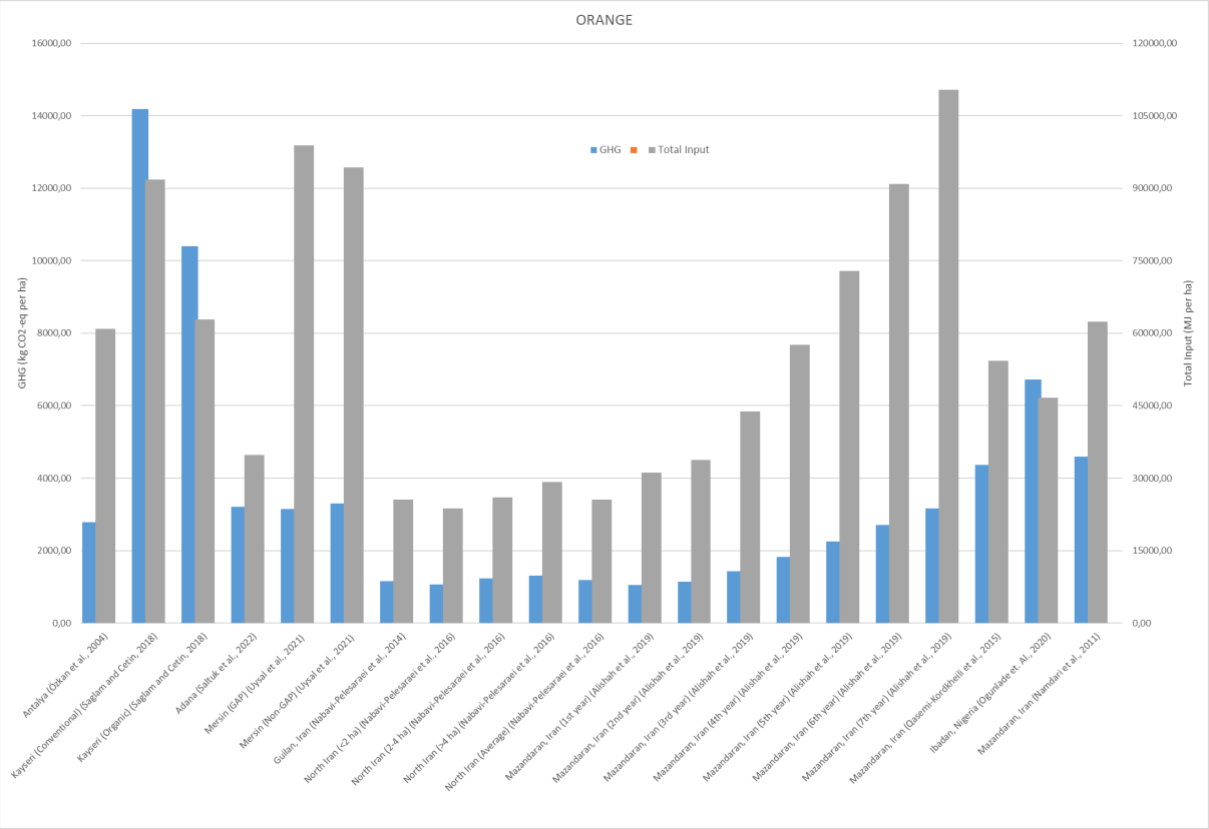

Figure 15. $CO_{2\text{-eq}}$ and total energy input values in orange production in the Iran and Turkey

Figure 16 shows the input percentage values for orange in the World. Figure 16 shows that Kayseri, which is a traditionally orange producing city in Turkey, has the highest share of chemical fertilizers (67.37%). The value of chemical fertilizers is 48.11%. The electricity value is very high in Adana (Turkey) with 62.60%. Human labor is mostly used in the harvesting process of orange production. Among these values, it is seen that, the human labor share is the highest in Ibadan (Nigeria) with a rate of 79.19%. The lowest rate of human labor is in Kayseri (Turkey) (Traditional) with 3.85%. It is seen in Figure that, the diesel input rate is highest in Mazandaran (Iran) (42.45%). The highest farm machinery input is in Guilan (Iran) with a rate of 13.15%.

The highest input rate in pesticide parameter is 29.95% in Kayseri (Turkey) (Organic). Insecticide use in orange production only in Turkey. The input rate is 1.73% and is highest in Mersin (Turkey) (Non-GAP). The rate of herbicide use in Mazandaran (Iran-1st year) is 9.11%. The energy input rate in fungicide use is 3.17% in Mazandaran (Iran-7th year). It is seen in Figure 16 that, the highest use of farmyard manure is in North Iran (<2 ha) (0.85%). It is seen in Figure 16 that, the input rate in nitrogen is 17.90% in North Iran (>4 ha). It is seen in Figure that, the input rate of electricity parameter has the highest rate with a value of 62.60% in Adana (Turkey). It is in the water parameter with a 46.60% input rate and it reached the highest value in Mazandaran (Iran).

Lemon

It can be seen in Figure 17 that the $CO_{2\text{-eq}}$ emission value is at the lowest in Antalya, Turkey (2655.32 kg $CO_{2\text{-eq}}$ per ha). The highest value is 3577.3 kg $CO_{2\text{-eq}}$ per ha occurred in Mersin (Turkey). Total energy input changed between 28952.2 in Mugla (Turkey) and 66741.2 MJ/ha in Mersin (Non GAP) (Turkey).

The input parameter rates for lemon production are given in Figure 18. Energy input analysis in lemon fruit was obtained by utilizing the data from the all published papers.

As seen in Figure 18, water, which is one of the input parameters, was used intensively in other cities except Antalya. It is seen in Figure 18 that, the input in human labor has the highest ratio in Adana (Turkey) with a value of 24.57%. The input rate in the diesel parameter is the highest share in Antalya (Turkey) (35.79%). It is seen in Figure 18 that, the input of farm machinery is concentrated in Mersin (Turkey) with a rate of 5.33%. The nitrogen fertilizer was used the most in Antalya (Turkey) (23.16%). The input parameter in electricity, with a rate of 51.92%, was used the most in Mugla (Turkey).

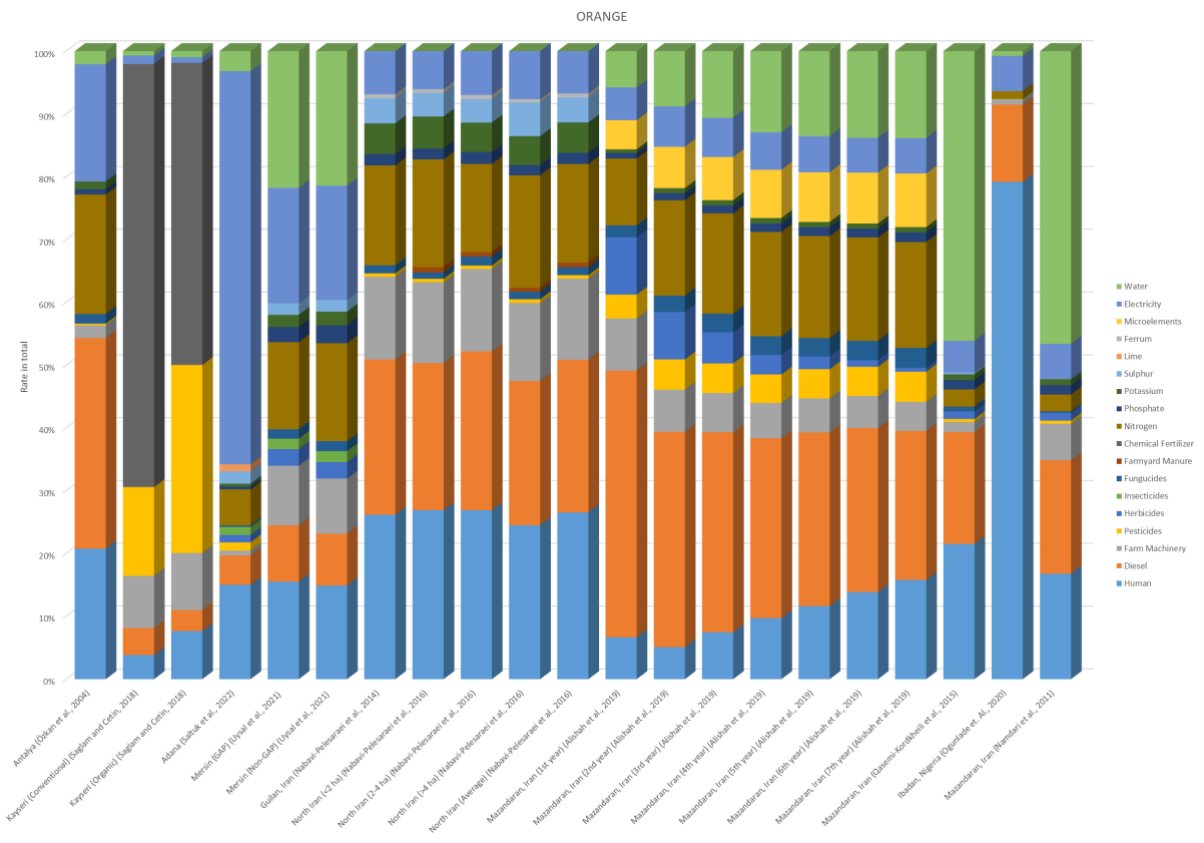

Figure 16. Percentage of input parameters in orange production in the Iran and Turkey

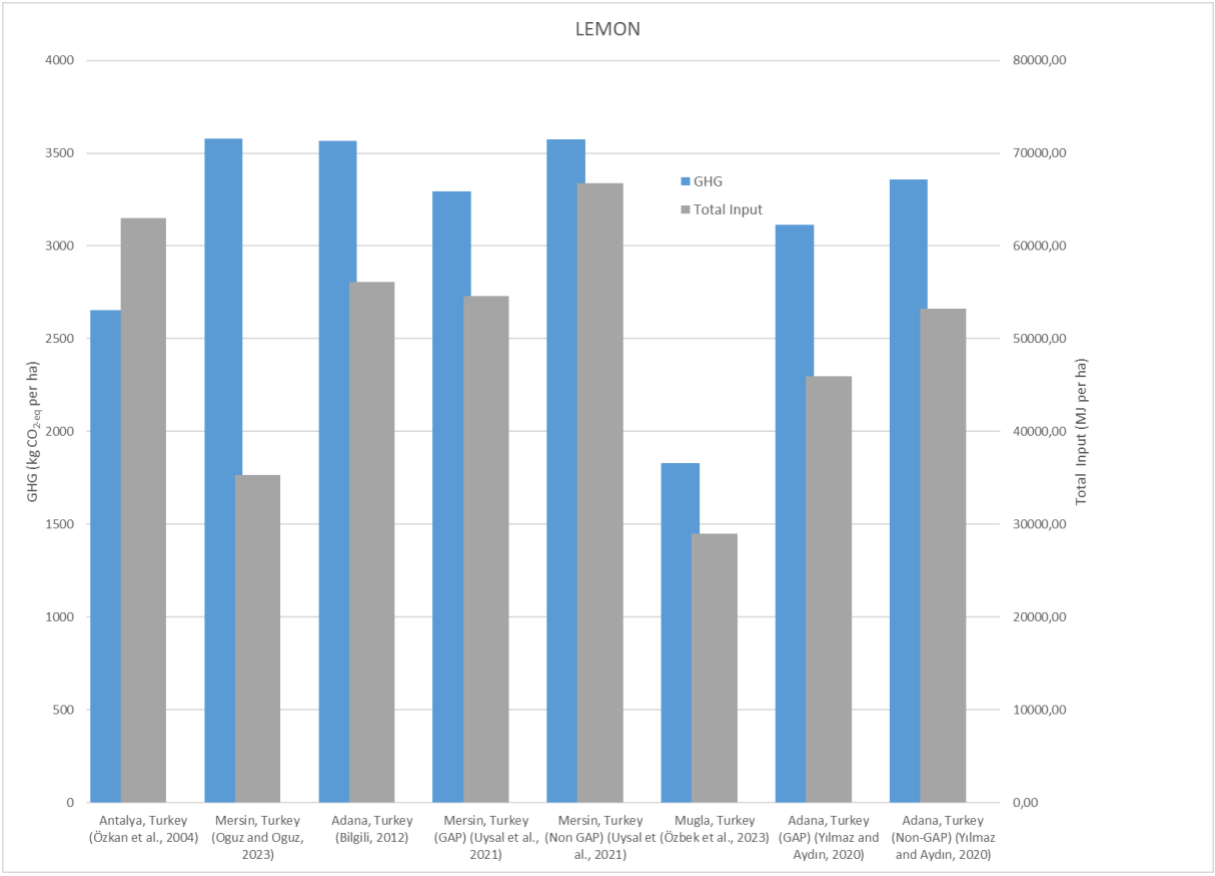

Figure 17. $CO_{2\text{-eq}}$ and total energy input values in lemon production in the Turkey

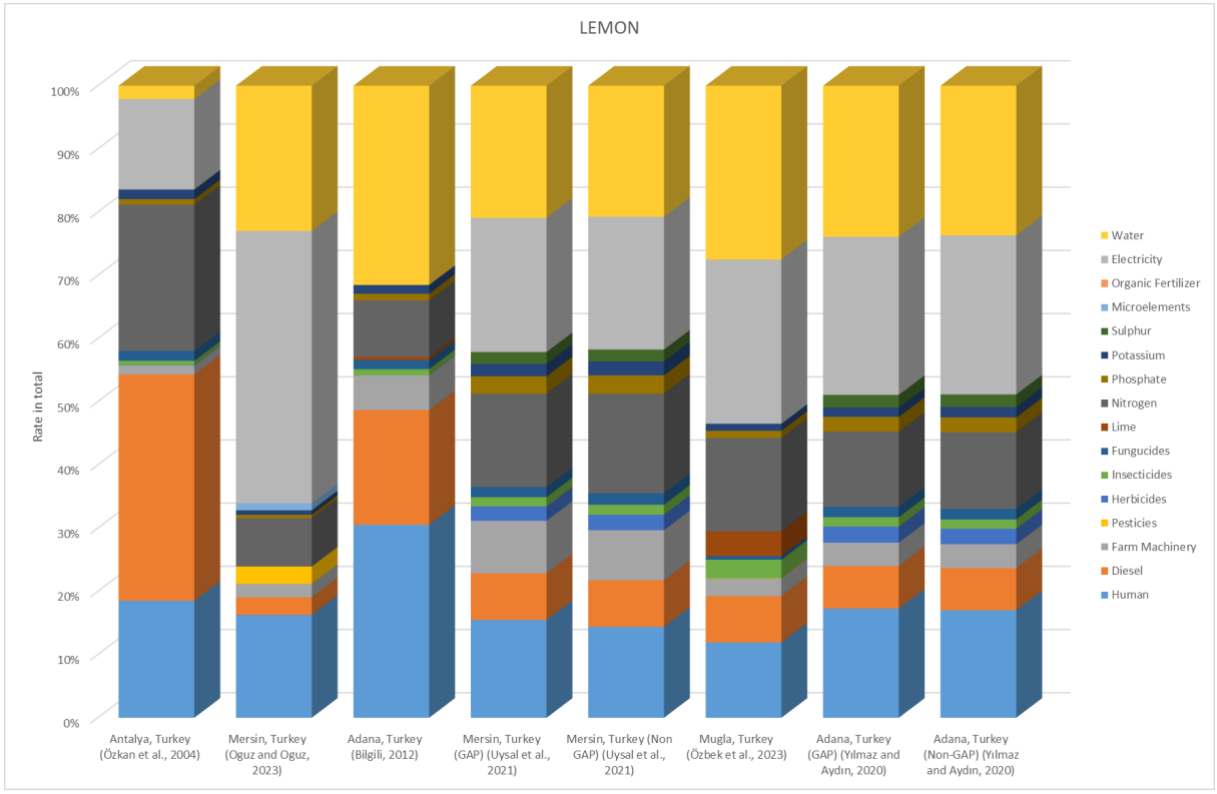

Figure 18. Percentage of input parameters in lemon production in the Turkey

Mandarin

Figure 19 shows $CO_{2\text{-eq}}$ per ha in mandarin production in the World. It is seen in Figure 19 that the highest $CO_{2\text{-eq}}$ value is in Mazandaran (Iran) with 5910.3 kg $CO_{2\text{-eq}}$ per ha. The lowest value is in Adana (Turkey) with 399.9 kg $CO_{2\text{-eq}}$ per ha. The total energy input is ranged between 4686.5 in Adana (Turkey) and 77501.2 MJ/ha in Mazandaran (Iran).

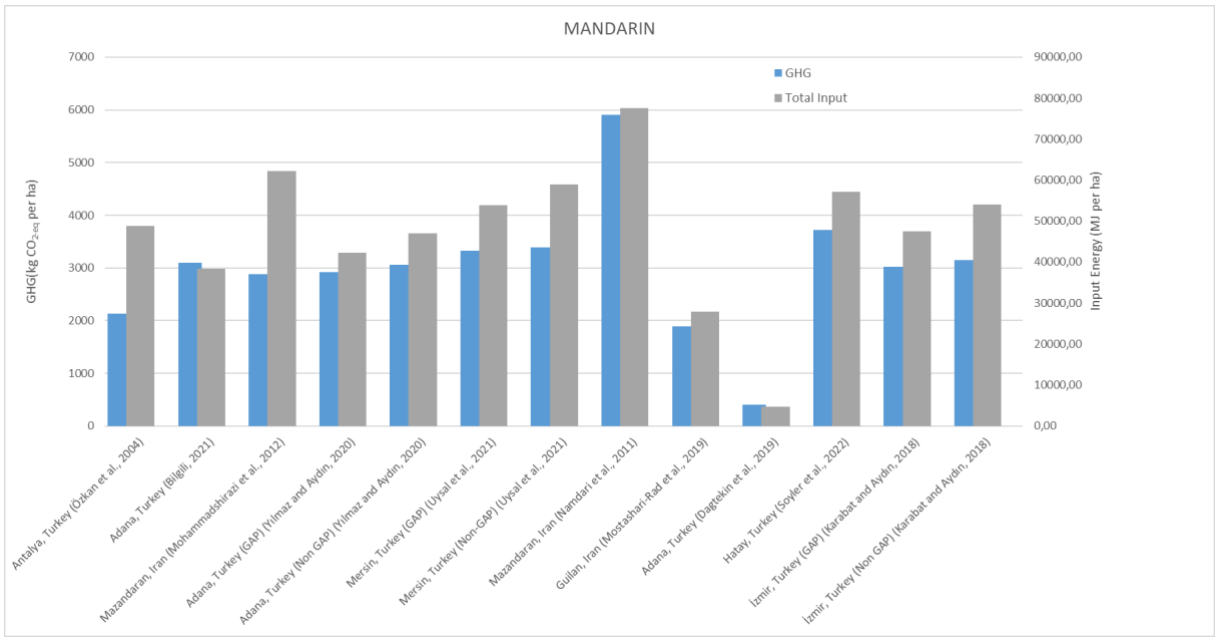

Figure 19. $CO_{2\text{-eq}}$ and total energy input values in mandarin production in the Iran and Turkey

Energy input rates in mandarin fruit produced in the world and in Turkey are given in Figure 20. A total of 15 input parameters and the necessary data were calculated.

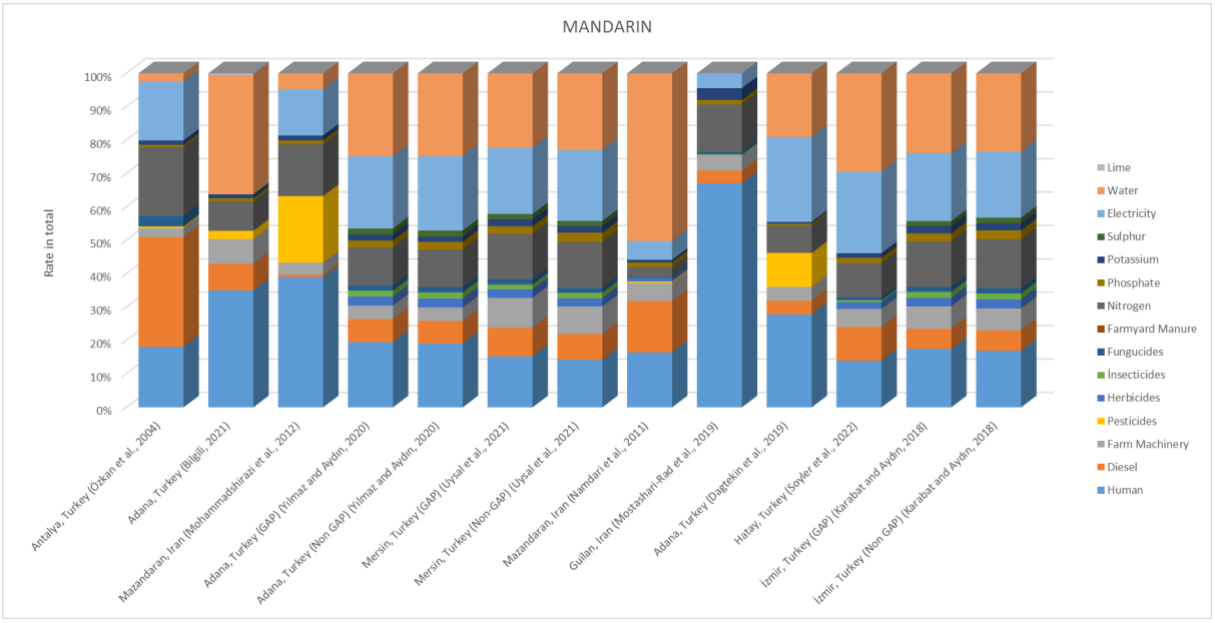

Figure 20. Percentage of input parameters in mandarin production in the Iran and Turkey

The human factor is of great importance in the harvesting of such fruits. In the harvesting process, which is the last stage of mandarin production in the World, more human labor is used. Therefore, the share of human labor is 67.09% in Guilan (Iran) as seen in Figure 20. Since the harvesting process of mandarin is based on human labor, input rates in farm machinery remain low. Looking at Figure 20, the highest input is in Mersin (Turkey) with a rate of 8.98%. It is seen in Figure 20 that, the input rate in diesel is 32.88% in Antalya (Turkey). Pesticide use was highest in Mazandaran (Iran) with a rate of 19.96%. As the rates of herbicide, insecticide and fungicide are given in Figure 20, the highest use is in Antalya (Turkey) with 3.06% fungicide, in Izmir (Turkey) with 2.69% herbicide (Non GAP) and in Adana (Turkey) with 1.86% insecticide (Non GAP). The use of farmyard manure in mandarin production is very low in the studies examined. Nitrogen fertilizer is used the most among the input rates in nitrogen, phosphate, potassium and sulphur fertilizers, with a rate of 20.59%, in Antalya (Turkey). The input rate of electricity is in Hatay (Turkey) with a rate of 24.43%. In the studies conducted in Mazandaran (Iran) in 2011 and 2014, it is seen in Figure 20 that, the input rate in water in mandarin production is 50.24%. Lime was used only in Adana (Turkey) (0.56%).

Grapefruit

Figure 21 shows the $CO_{2\text{-eq}}$ input values in grapefruit production in the World. $CO_{2\text{-eq}}$ values are seen in Figure 21 as 3945 kg $CO_{2\text{-eq}}$ per ha in Hatay (Turkey), 3130.0 kg in the USA and 1868.7 kg $CO_{2\text{-eq}}$ per ha in the USA. Total energy input is 60944.9, 53083.6 and 31612.5 MJ/ha, respectively, in the same locations.

Grapefruit is a fruit in the citrus family. As seen in Figure 22, energy analysis values in grapefruit were calculated on 3 different studies and with 14 input parameters.

Figure 22 shows the input rates in grapefruit produced in Turkey and the World. Grapefruit production was carried out in Hatay (Turkey) and the United States. The rate of human labor, which is one of the input parameters, calculated as 16.21% in Hatay (Turkey). In developed countries where fuel is not an economic problem, such as the USA, input rates are also high. The highest diesel consumption is in the USA with a rate of 45.85%. The input rate in farm machinery use is seen in Hatay (Turkey) with a rate of 4.73% in Figure 22. Although no pesticides were used in Hatay (Turkey), they were used in the USA with a rate of 18.12%. The input rates of herbicides, insecticides and fungicides from the energy input parameters are shown in Figure 22. Insecticide was used only in Hatay (Turkey) with a rate of 1.57%. Herbicide is at its highest value in Hatay (Turkey) (1.74%). Fungicide is in the USA with a rate of 1.17%. Among the chemical fertilizers used in grapefruit production are nitrogen, phosphate and potassium. With a rate of 13.64% in nitrogen fertilizer, input parameter was used more in the USA. Although the input rates in phosphate and potassium are close to each other, the highest phosphate input is in Hatay (Turkey) with a rate of 1.47% and the highest potassium input is in the USA with a rate of 2.10%. Although lime is not used in Hatay (Turkey), it is seen in Figure that it is used the most in the USA with a rate of 23.37%. Electricity and water were used only in Hatay (Turkey) with a value of 26.48% and 24.45%, respectively.

Maximum and minimum GHG emission values are given in Figure 23. The fruit with the highest GHG emission value is strawberry (34517.75 kg $CO_{2\text{-eq}}$ per ha). After strawberry fruit, plum (31109.13 kg $CO_{2\text{-eq}}$ per ha), wolfberry (20718.66 kg $CO_{2\text{-eq}}$ per ha) and orange (14183.87 kg $CO_{2\text{-eq}}$ per ha) fruits come respectively. The fruit with the lowest GHG emission value is mandarin (399.89 kg $CO_{2\text{-eq}}$ per ha). There are big differences between

minimum and maximum emission values in different regions or countries. Because different practices are applied during production in each region or country. In fact, while no fertilizer is used in one location, all types of fertilizers can be used in another location. This difference in inputs also affects the total greenhouse gas emission values produced.

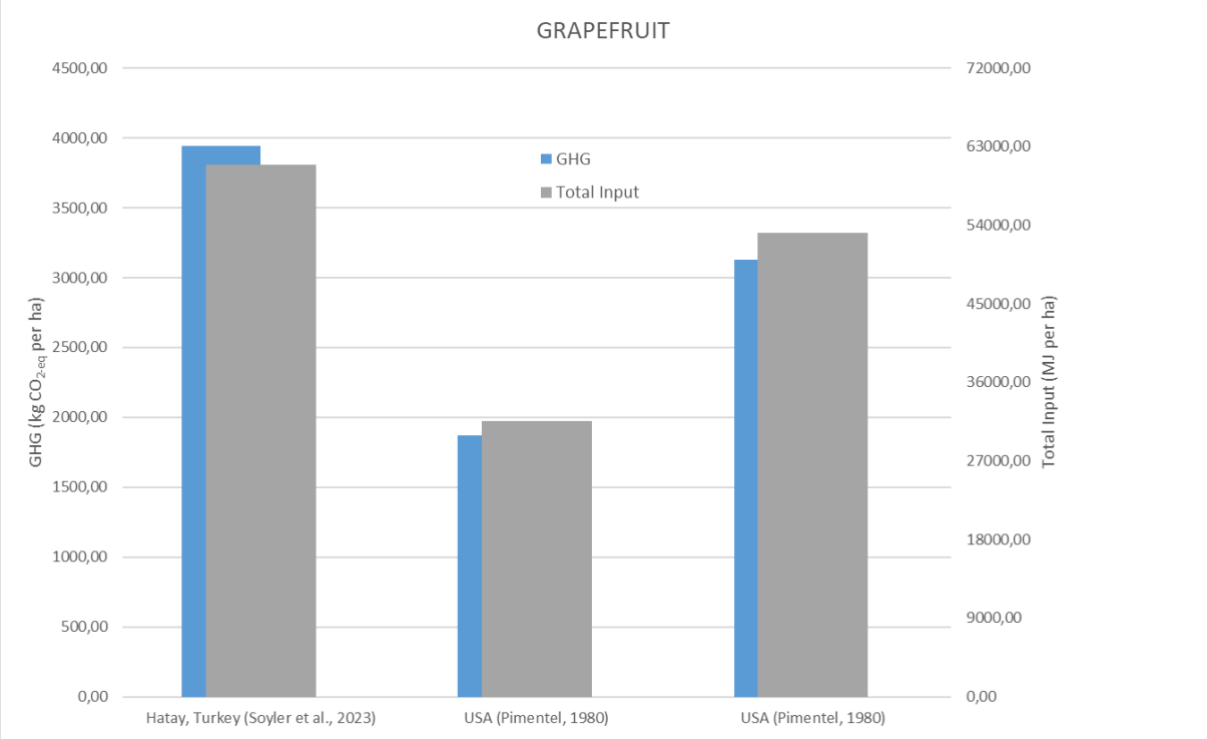

Figure 21. $CO_{2\text{-eq}}$ and total energy input values in grapefruit production in the USA and Turkey

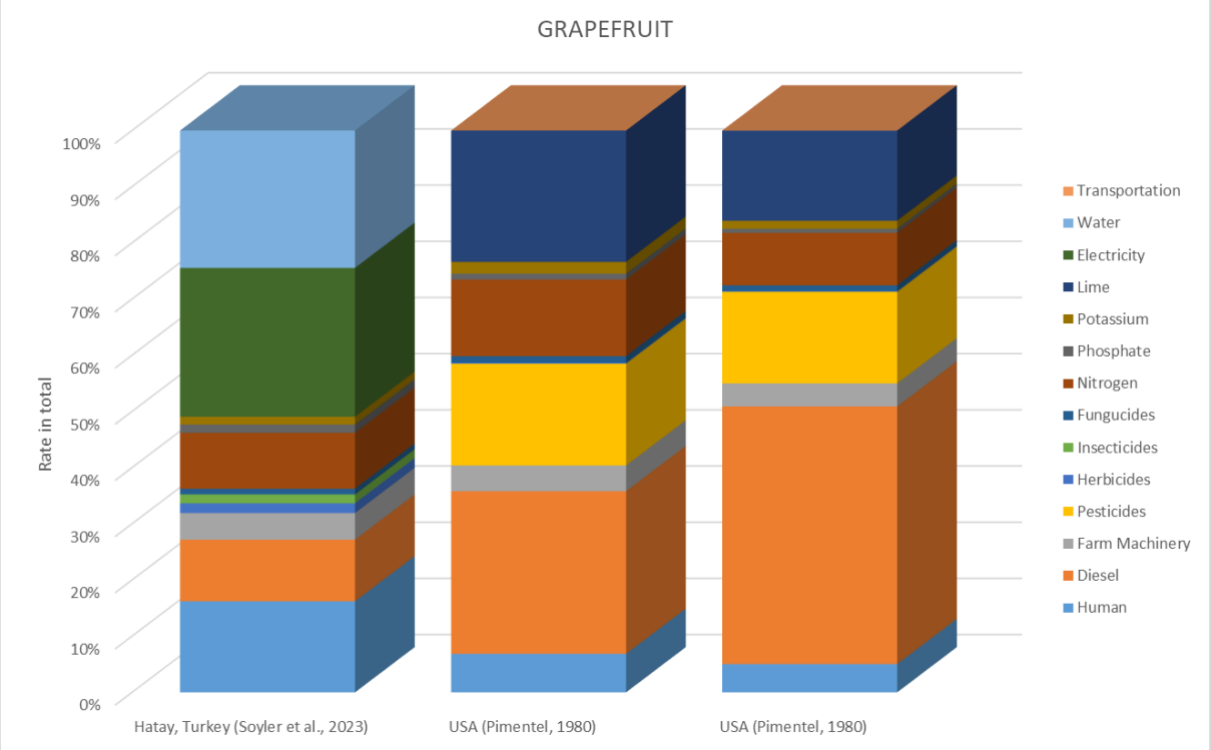

Figure 22. Percentage of input parameters s in grapefruit production in the USA and Turkey

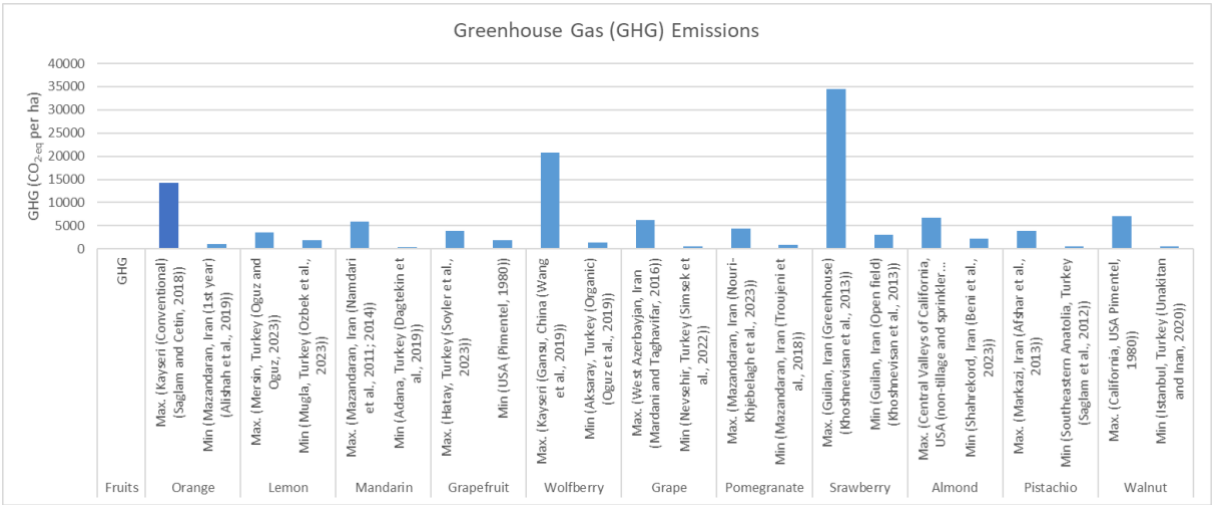

Figure 23. Maximum and minimum Greenhouse Gas (GHG) emission values

CONCLUSIONS

Global warming and climate changes occurring on earth pose a threat to living and non-living beings. The human factor is of great importance in this regard among living beings on Earth. In addition, the amount of greenhouse gas emissions does not positively affect global warming and climate change. The amount of greenhouse gas emissions generated in agricultural processes is also not negligible. Under the headings of berry, nut and citrus fruit; greenhouse gas emission values in grape, pomegranate, strawberry, wolfberry almond, pistachio, walnut, orange, lemon, mandarin/tangerine and grapefruit were calculated. GHG value reached the highest amount in strawberry fruit, while mandarin fruit has the lowest greenhouse gas emission value. The biggest part of the emissions comes from chemical fertilizers, pesticides, fuels and human. So, these input parameters usage could be reduced to a reasonable level with high yield. It contributes to agriculture against the harmful effects of greenhouse gas emissions. Renewable energy carbon footprint is positively affected by greenhouse gas emission values. It also provides financial support to farmers. Minimum tillage processing could be adviced to reduce CO_{2-eq} values. Reducing ferlizer usage and especially avoiding excess nitrogen usage after appliying soil analysis can improve in reducing CO_{2-eq} values. Low carbon farm machinery usage and improved human labor efficiency could have other alternatives to reduce GHG emissions.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Authors' individual contributions to the article are equal.

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Investigation of the antimicrobial activity of water and methanol extracts of *Salvadora persica L.* (Miswak) plant against some pathogenic microorganisms

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DergiPark AKADEMİK

Abstract

The toothbrush tree *Salvadora persica L*, also called miswak, belonging to the family *Salvadoraceae*, is one of the most important of the 182 plant species used as chewing sticks. It is widely used in many Asian, African and Middle Eastern countries. The roots, branches and stems of this plant have been used for oral hygiene and small miswak sticks have been used as toothpicks for oral hygiene. In this study, commercially purchased *Salvadora persica*, L. (Miswak) plant used in oral hygiene were tested against seven pathogenic bacteria (*Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853) and two fungi (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) at eight different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12. 5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml) were determined. While the aqueous extract did not show any antimicrobial activity against seven pathogens, the methanol extract showed activity against three pathogens. The methanol extract of *S. persica* showed antimicrobial activity against *Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623 and *Klebsiella pneumoniae* ATCC 70060 strains.

Keywords: *Salvadora persica*, Methanol, Antimicrobial Activity, Micro-dilution, Disc Diffusion

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INTRODUCTION

The emergence of multidrug-resistant human bacterial pathogens in the 1990s, and more recently of widely resistant clinical isolates, has hampered efforts to control and manage human infections caused by these organisms (Magiorakos et al., 2012). The development of antimicrobial resistance due to the misuse of antibiotics has become a matter of concern (WHO, 2015). Furthermore, the continuous increase in global isolation rates of clinical isolates such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and carbapenem-resistant Gram-negative bacilli poses a serious therapeutic challenge, as no new antimicrobial agents are currently available for the treatment of infected patients (Elabd et al., 2015; Asaad et al., 2013; Al-Ayed et al., 2016).

Studies on the antibacterial properties of *Salvadora persica* (miswak) show that especially water and methanol extracts are effective against various pathogens. For example, one study found that miswak extracts exhibited moderate to strong antibacterial activity against multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (Al-Ayed et al., 2016).

Similarly, there are studies on the antibacterial activities of different plant and fungal species. For example, the antimicrobial effects of methanol and ethanol extracts of *Ramaria aurea* mushroom against MRSA and other pathogens were investigated and found to be effective against some strains (Güneş and Alkan, 2024).

Plants are important in human life and meet the daily needs of people. They are used as cosmetics, food, flavourings, ornaments and medicines. Medicinal plants have become part of complementary medicine worldwide

due to their potential health benefits. Various plant extracts have great potential against infectious agents and can be used for therapeutic purposes (Upadhyay et al., 2010; Gomez-Flor et al., 2006).

The toothbrush tree *Salvadora persica L*, also called miswak, is widely used in many Asian, African and Middle Eastern countries. In studies, it has been determined that the roots, branches and stems of *Salvadora persica L* plant are used for oral hygiene and small miswak sticks are used as toothpicks to ensure oral hygiene (Sher et al., 2011). Water and methanol extracts of miswak have been reported to 4have various biological properties against organisms thought to be important in the development of dental plaque and periodontitis (Sofrata et al., 2008).

Previous in vitro studies have shown that miswak is effective against *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa, Aggregatibacter actinomycetemcomitans,* They reported antibacterial and antifungal effects on cariogenic bacteria and periodontal pathogens including *Porphyromonas gingivalis, Haemophilus influenzae* and *Candida albicans* (Naseem et al. , 2014; Al-Sieni, 2014; Al-Bayati and Sulaiman, 2008; Alireza et al., 2014; Fallah et al., 2015; Alili et al., 2014; Mohammed, 2013; Al-Ayed et al., 2016).

A literature survey shows that much effort has been devoted to the study of the inhibitory effect of *Salvadora persica* on oral organisms, but there is little information on the antibacterial and antifungal activity of *Salvadora persica* against other human pathogens. In this project, the antibacterial and antifungal activity of water and methanol extracts of *Salvadora persica* against seven pathogenic bacteria and two pathogenic fungal species was investigated. While the water extract did not show any antimicrobial activity against seven pathogens, the methanol extract showed activity against three pathogens.

MATERIALS AND METHODS

Commercial Supply of *Salvadora persica L.* (Miswak) Plant

The powder form of *Salvadora persica* plant was purchased from Aktarloji Natural Products company and stored at room temperature in a cool and dry place until the study.

Preparation of *Salvadora persica* Extracts

Aqueous Extracts (H_2O)

40 g of *S. persica* (miswak) plant powder was taken, 200 ml of distilled water was added and homogeneous mixing of water and plant extract was ensured in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuum, dried at 60°C with a rotary evaporator and diluted with 25% Dimethyl sulfoxide (DMSO) for antimicrobial activity study (Kandil et al., 1994).

Methanol Extracts (MeOH)

200 ml of distilled water was added to 40 g of finely powdered *S. persica* (miswak) and a homogeneous mixture of water and plant extract was provided in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the solvent was removed using a rotary vacuum evaporator at 40°C, the concentrated extract was obtained and the dilution required for the antimicrobial activity study was made with 25% Dimethyl sulfoxide (DMSO) (Hassanin et al., 2020, Saad et al., 2020).

Determination of In Vitro Antimicrobial Activity

Microorganism

Pathogenic microorganisms used in this study were obtained from the American Type Culture Collection. Gram-positive bacteria (*Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853), Fungi (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) were used in the antimicrobial activity study.

Disc Diffusion Method

The antimicrobial activity of *S. persica* (miswak) was evaluated using the disc diffusion method (Bauer et al., 1966). Muller Hinton Agar (MHA) was used to activate bacterial cultures and Sabouraud Dextrose Agar (SDA-Difco) was used for fungi. Before the study, microorganisms were transferred to Muller Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB-Difco) for fungi and allowed to grow overnight at 37°C (28°C for fungi). The turbidity of the prepared suspensions of the test strains was adjusted to 0.5 McFarland equivalent (1.5×10^8 cfu/ml) and 100 µl of pathogenic microorganisms were spread on the surface of the agar plate with sterile swabs and allowed to dry in Laminar Flow for five minutes. *S. persica* (miswak) extracts (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml) obtained using solvents (water and methanol) were mixed with 1 ml of 25% Dimethyl sulphoxide (DMSO). Filter paper discs (6 mm) were impregnated with 25 µl of the extracts to check their antimicrobial activity and allowed to dry in Laminar Flow. After drying, the plates with bacteria were incubated overnight at 37°C and the plates with fungi were incubated at 28°C. 25% DMSO was used as a blind control, Amphicillin (AM10), Polymyxin B (PB300) and Nystatin (Cyc) (for fungi) were used as positive controls. The zone of inhibition was observed and measured in millimetres. The study was repeated three times and the results were averaged.

RESULTS AND DISCUSSION

Commercially available powdered form of *S. persica* plant was taken 40 g, 200 ml of distilled water was added and homogeneous mixing of the plant extract with water and methanol was achieved in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried with a rotary evaporator at 60°C for water and 40°C for methanol and the necessary dilution for antimicrobial activity study was made with 25% Dimethyl sulfoxide (DMSO) (Kandil et al., 1994). A schematic visualisation of the extraction process is given in Figure 1.

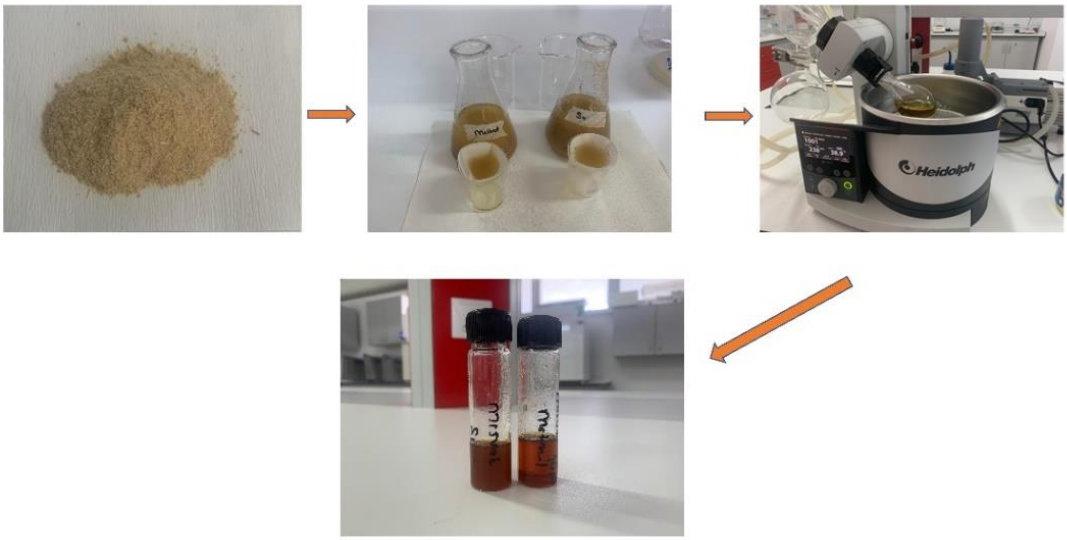

Figure 1. Schematic image of the preparation of *Salvadora persica* extracts

Water and methanol extracts of *S. persica* (miswak) were inoculated on discs at eight different concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml.

The antibacterial activities of water and methanol extracts of *S. persica* against 4 gram-positive, 3 gram-negative and 2 pathogenic fungi are listed in Table 1. In general, the methanol extract of miswak showed activity against three of the tested pathogenic microorganisms (Figure 2), while no concentration of water extracts showed growth inhibitory effect (Figure 3).

Table 1. Antimicrobial activity of *Salvadora persica* extracts prepared with water and methanol solvents.

Microorganisms	Concentrations of *Salvadora persica* extracts (mg/mL) and inhibition zones (mm)																		
	Water								Methanol								Positive control		
	200	100	50	25	12.5	6	3	1	200	100	50	25	12.5	6	3	1	Ampicillin (AM10)	Polymixin B (PM300)	Nystatin (NS100)
Bacillus cereus	-	-	-	-	-	-	-	-	10	10	9	9	9	-	-	-	30	10	-
Bacillus subtilis	-	-	-	-	-	-	-	-	12	10	10	9	9	8	-	-	30	10	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	10	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	10	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	15	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	10	9	9	-	-	-	-	-	40	20	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	15	-
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30

When the inhibition zone diameters are evaluated, it is seen that the methanol extract of *S. persica* is effective against members of the genus *Bacillus* from gram positive bacteria. It formed an inhibition zone between 10 mm and 9 mm on *B. cereus* bacteria. The lowest effective concentration is 12.5 mg/ml. *B.subtilis* bacteria formed an inhibition zone between 12 mm and 8 mm. The lowest effective concentration was 6 mg/ml. *K. pneumoniae*, another pathogenic bacterium, formed a zone of inhibition between 10 mm and 9 mm and showed the lowest effect at a concentration of 50 mg/ml (Figure 2).

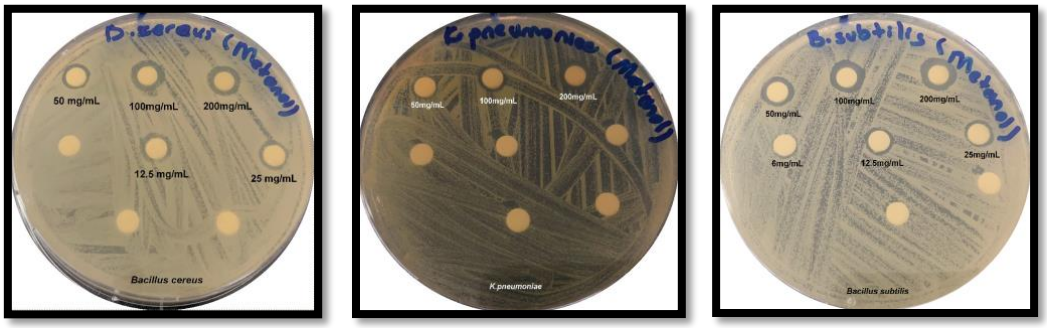

Figure 2. Inhibition zones created by the extract obtained from *Salvadora persica* with methanol solvent against *Bacillus* genus members and *K.pneumoniae* pathogen

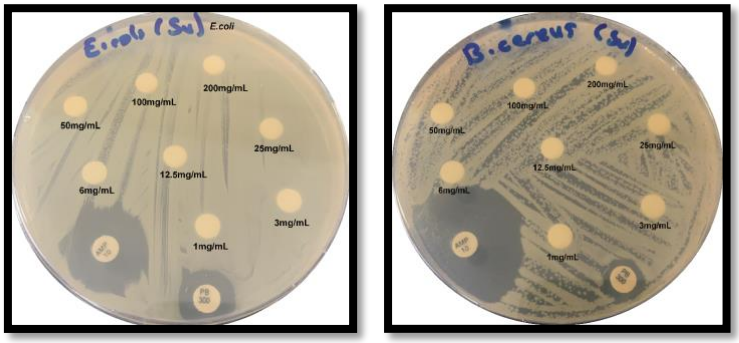

Figure 3. Petri dish image in which the extract obtained from *Salvadora persica* with water solvent has no effect on *E.coli* and *B.cereus* pathogens

When compared with the standard antibiotics used, the extract obtained from methanol exhibited antimicrobial activity lower than amphicillin and polymyxin B standards at a concentration of 200 mg/ml against members of the genus *Bacillus*. The methanol extract showed lower activity against *K. pneumoniae* pathogenic bacteria than polymyxin B antibiotic.

Al-Ayed et al. (2016) evaluated the in vitro antibacterial activity of *S. persica L.* extracts against 10 MDR (MRSA, MRSE, *Streptococcus pyogenes*, *E. faecalis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. marcescens*, *A. Baumannii* and *S. maltophilia*) bacterial clinical isolates other than oral pathogens. The antibacterial activity of water and methanol miswak extracts was evaluated using agar dilution and minimum inhibitory concentration (MIC) methods. In general, 400 mg/mL miswak extracts were found to be the most effective in all strains. Methanol extract showed a stronger antibacterial activity against Gram-negative (3.3-13.6 mm) bacteria than Gram-positive (1.8-8.3 mm) bacteria. The lowest MIC value was observed for *E. coli* (0.39, 1.56 μg/mL), followed by *Streptococcus pyogenes* (1.56 μg/mL). The highest MIC values (6.25, 12.5 μg/mL) were recorded for methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*.

Apaydın (2018) investigated the antioxidant and antimicrobial effects of essential oil obtained from *S. persica* (miswak) plant. For this purpose, 250 grams of powdered miswak sample was extracted by hydrodistillation method. Antimicrobial properties of essential oils were determined by disc diffusion method according to the method of Parvathy et al. They found that miswak oil showed antimicrobial activity against *S. aureus* and *E. coli* bacteria by forming a zone of 4 and 18 mm in diameter, respectively.

Another study was conducted by Al-Sieni in 2014. He collected and dried *Salvadora persica* (miswak) and *Commiphora gileadensis* plants and evaluated the extracts extracted with methanol and warm water for their antibacterial activity against 5 different bacterial genera (*Fusobacterium nucleatum, Lactobacillus casei, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans* and *Streptococcus salivarius*) using agar well diffusion method. Some human pathogens were among the tested bacteria. The obtained extracts showed significant inhibitory effects against all tested bacteria with various degrees of growth inhibition. Methanol extracts were more effective than water extracts. The minimum inhibitory concentrations (MIC) of the methanol extracts

ranged from 50-100 µg/ml. In conclusion, *S. persica* and *C. gileadensis* showed moderate to high inhibitory activity on pathogenic bacteria and can be used traditionally in alternative medicine.

Among the reasons for the difference between the results obtained in this study and the results of other researchers, miswak extracts may exhibit different antibacterial and antifungal activity due to reasons such as the method of obtaining the extract, the components in the chemical content of the extract, solvent diversity, the type of pathogenic microorganism used or the use of different strains of the same species (Elnabris et al., 2013; Ramalingam and Amutha, 2013; Gümüş and Ünlüsayın, 2016).

CONCLUSION

Until now, many different studies have been carried out on extracts and oil of *S.persica* plant and it has been observed that they show various activities against Gram-positive, Gram-negative and fungi. This makes *S. persica* plant especially important in terms of having the potential to be used in the field of health.

In conclusion, the methanol extract of *S. persica* exhibited antibacterial activity against three pathogens and further in-depth studies should be carried out to isolate the active constituents involved in this activity.

Some studies show that plant-based antimicrobials may offer potential treatment options, especially against pathogens that show antibiotic resistance. Therefore, the development and use of plant-based antimicrobials is considered as an important step in the fight against antimicrobial resistance.

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 table are original and that they have not been published before.

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Effects of temperature rise on grapevine phenology (*Vitis vinifera* L.): Impacts on early flowering and harvest in the 2024 Growing Season

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Abstract

Numerous studies have documented that climate change will considerably impact grapevine phenology. In the 2024 growing season, notable differences emerged from the previous five years, between 2019 and 2023. Rising temperatures at the beginning of the growth cycle of grapevines began the phenology phases earlier, leading to earlier bud burst and flowering for various grape varieties in many regions of Türkiye. This study examines variations in phenological phases across different years, using short-term climate data from a weather station in a cv. Sultan 7 (*Vitis vinifera* L.) vineyard in Yunusemre, Manisa, Türkiye. The objective of the study is to determine the impact of temperature fluctuations ranging from January to September on grapevine phenology intervals during critical stages: bud burst, full bloom, veraison, and maturity, specifically for between 2019 and 2024. Evaluations focused on critical factors, including growth cycle duration, days within specific temperature ranges, effective heat summation for the variety, and the Winkler Index values. The 2024 growing season recorded the highest temperatures in April and June, and bud burst occurred 5 days (2021) to 10 days (2019) earlier, while full bloom was determined for 16 days (2019 and 2022) to 27 days (2021) earlier than in previous years. The findings showed that years characterized by earlier flowering, 2024 (day of the year (DOY) 120) and 2022 (DOY 136), may be associated with earlier harvest. In addition, the Winkler Index recorded a highest of 2945.01 growing degree days (GDD) in 2024, with a specific effective heat summation value of 2,138.79 GDD for variety in a shorter timeframe. The findings suggest that although the intervals between veraison and harvest tend to remain almost similar each year, early flowering, the ripening period, and elevated temperatures before veraison in the same season can greatly contribute to prompting an earlier harvest.

Keywords: Bloom, Phenology, Heat requirement, Grape, Climate change, Vineyard

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INTRODUCTION

Many studies from around the globe have underscored the effects of climate change on viticulture. Particularly, rising temperatures significantly affect the phenology (Fraga et al., 2012; Munoz-Organero et al., 2022; Cameron et al., 2022; Ramos et al., 2023; Parker et al., 2024; Espinosa-Roldán et al., 2024) and physiological responses of grapevines (Bartlett and Sinclair, 2021; Arias et al., 2022; Wu et al., 2023), as well as vine yield and grape quality (Rogiers et al., 2022; Rafique et al., 2023; Teker 2023; Teker and Soltekin, 2023).

Previous research indicates that the impact of climate change on grapes and their phenological cycles will differ based on location and cultivar (Jones et al., 2012; Arias et al., 2022; Parker et al., 2024). The relationship between grapevine phenology and climate variables differs in various phenophases. Moreover, maximum temperatures strongly influence early season events, such as bud break and bloom. In contrast, for later season events like veraison and harvest, average temperatures, growing degree-days (Winkler Index), and Huglin index values play a more significant role (Dalla Marta et al., 2010; Alikadic et al., 2019). On the other hand, rising temperatures

have been associated with an advance of 6 to 25 days in the phenological stages of various grape varieties across Europe. On average, this shift corresponds to a response of 3 to 6 days for each 1°C increase in temperature observed over the past 30 to 50 years (Jones et al., 2005; Jones, 2007). Moreover, a recent study in Italy by Alba et al. (2024) indicates that wine regions must adjust to considerably warmer climate conditions, which may impact the quality and distinctiveness of the wines produced.

During the 2024 growing season, many regions worldwide faced record-high temperatures in April. The global surface air temperature reached 15.03°C, 0.67°C above the 1991-2020 average and 0.14°C higher than the previous April record of 14.89°C set in 2016. In Europe, April 2024 was 1.49°C warmer than the 1991-2020 average, marking it the continent's second-warmest April (Copernicus Climate Change Service [CCCS], 2024). Similarly, analysis indicates that April and June are the hottest months in Türkiye over the past 53 years. Average temperatures increased from 12.3°C in April and 21.8°C in June between 1991 and 2020 to 16.6°C and 25.4°C, respectively, in 2024 (TMS, 2024a; 2024b). This phenomenon also impacted various viticultural regions in Türkiye during key phases of grapevine growth, particularly at the onset of growth in April and during the berry set in June. Consequently, this unusual climatic occurrence is believed to have led to an earlier-than-expected harvest time of many varieties in 2024.

This study examined the changes in phenological phases of the grape variety cv. Sultan 7 (*Vitis vinifera* L.) has been widely cultivated in the Aegean Region in the western part of Türkiye over the past six years (2019-2024). This study focuses on three main questions: 1) What minimum, average, and maximum temperatures were recorded from January to September (from a near-dormant phase to the time of harvest) between 2019 and 2024? 2) How did the variations between the years impact the grapevine growth cycle and phenological phases? 3) How was the variation in effective heat summation, a measure of cumulative growing degree days from budburst to harvest for the grape cultivar studied, and how did the temperatures influence the Winkler Index?

MATERIALS AND METHODS

Study area and plant material

Temperature data, including minimum, mean, and maximum values, were collected monthly and daily from 2019 to 2024 from a weather station (iMETOS IMT 280, Pessl Instruments, Weiz, Austria) situated in Yunusemre (YE) [38°37′ N, 27°24′ E; Altitude (A): 44 m], which is centrally located in the Manisa province of western Türkiye. Due to the unavailability of data for October 2024 from the Yunusemre station, information was obtained from the Muradiye station (38°41′ N, 27°22′ E; A: 23 m), located almost 6.37 kilometers away from the YE station. Manisa city center generally has a warm-temperate climate, with recent hot and dry summer seasons and limited rain (Teker and Altındişli, 2021). However, the average annual temperatures are 16.9 ℃ with 743.6 mm of precipitation per year according to the long-term regime from 1930-2023 (TMS, 2024c). As a plant material, cv. Sultan 7 (*Vitis vinifera* L.) cultivar was used to understand the length of the vegetation period and emphasize the phenological phase differences between years.

Determination of phenological stages in cv. Sultan 7 (*Vitis vinifera* L.)

To assess the phenological stages of cv. Sultan 7 between 2019 and 2024, the modified Eichhorn & Lorenz (EL) system, as outlined by Coombe (1995), was employed. This approach allowed for the precise determination of key stages, including bud burst (EL 4), full bloom (EL 26), veraison (EL 35), and harvest (EL 38). The vegetation period length was also determined using these terms in each year.

Seasonal temperature ranges and variations (2019-2024)

Minimum, mean, and maximum temperatures were recorded daily between 2019 and 2024. The number of days with temperatures below 0°C (T°C ≤ 0°C), as well as those within the ranges of 0°C to 5°C, 5°C to 10°C, and 10°C to 20°C, specifically during January, February, March, and April was determined. Maximum temperature ranges were analyzed, focusing on the number of days with increasing temperatures from April to September. This evaluation encompassed the onset of the growth cycle and the period following the harvest. These ranges included 10°C to 25°C, 25°C to 30°C, 30°C to 35°C, 35°C to 40°C, and temperatures exceeding 40°C (where T°C is greater than or equal to 40). April 2024 was included in both evaluations due to its notable differences from the other years under consideration. The variations in these temperature ranges across different years were then depicted in graphs.

The determination of heat summation as growing degree days for studied cultivar

The phenological observation dates established for the cv. Sultan 7 grape variety were utilized to calculate heat summation as Growing Degree Days (GDD). Cumulative values of GDD were determined based on the specific phenological stages of the variety, which include the periods from bud burst to full bloom, from full bloom to veraison, and between veraison and harvest in the vegetation period of grapevines. The cumulative GDD formula employed in these calculations is presented in Equation 1.

$$\sum_{Bud\ Burst}^{Full\ Bloom}\left(\frac{Tmax+Tmin}{2}-10℃\right)+\sum_{Full\ Bloom}^{Veraison}\left(\frac{Tmax+Tmin}{2}-10℃\right)+\sum_{Veraison}^{Harvest}\left(\frac{Tmax+Tmin}{2}-10℃\right)=\sum_{Bud\ Burst}^{Harvest}\left(\frac{Tmax+Tmin}{2}-10℃\right) \quad (1)$$

Winkler Index (WI)

The WI was determined as GDD by summing the total degrees of mean daily temperatures exceeding 10°C for each day during the vegetation period, which was conducted from April 1 to October 31 across the years 2019 to 2024 (Winkler, 1974). Differences were revealed between years. The formula utilized for these calculations is provided in Equation 2.

$$WI = \sum_{April\ 1}^{October\ 31} \left(\frac{Tmax + Tmin}{2} - 10°C \right) \quad (2)$$

Statistical analysis and evaluation data

From January to October, the average, maximum, and minimum temperatures were analyzed monthly using statistical methods. The temperature values underwent a Shapiro-Wilk normality test. As a result, differences between 2019 and 2024 are presented as monthly comparisons based on a one-way ANOVA test accompanied by Tukey's multiple comparison test. Furthermore, the number of days within specific monthly temperature ranges was calculated, and these results were presented as raw data. The cumulative GDD values, Winkler Index calculations, vegetation period length, and phenological phases for each year were presented as raw data, and the differences were represented in a graph.

RESULTS AND DISCUSSION

Weather conditions of the study area and phenological phases

This study investigates the reasons behind the earlier grape harvest in Türkiye in 2024 compared to the previous five years (2019-2023) and a change driven by rising temperatures, a trend observed in many regions worldwide. Furthermore, the study explores the relationship between temperature fluctuations and the phenological changes that occur throughout the growing seasons of the relevant years.

According to the monthly bulletins from the Copernicus Climate Change Service, average temperatures recorded in 2024 have set new national and global records compared to values from 1991 to 2020. Notably, February, April, and June have experienced unprecedented temperatures (CCCS, 2024). In Türkiye, April and June in 2024 have also marked the highest temperatures in the past 53 years (TMS, 2024a; 2024b). Previous research has shown a notable advancement in the timing of three critical phenological phases of grapevines: flowering, veraison, and maturity (Cameron et al., 2022). Studies suggest that temperature is a key factor driving these changes in phenological phases (Bock et al., 2011; Ramos et al., 2023; Parker et al., 2024). As a result, there have been significant shifts in the timing of grape harvests compared to previous years, with many regions completing their harvests earlier in 2024.

Assessment of variations in monthly temperatures (2019-2024)

Figure 1 illustrates the variations in the monthly mean values of minimum, average, and maximum temperatures from January to September between 2019 and 2024. Notably, during the months that are crucial for the growing season and vegetative development of grapevines, particularly February (Figure 1B), March (Figure 1C), April (Figure 1D), and June (Figure 1F) in 2024, the average and maximum temperature values, monthly for 2024 were notably higher than those recorded in the preceding five years. In the western region of Türkiye, specifically in Manisa, the elevated average temperatures recorded in March and April were critical in determining the beginning of the grapevine growth period, as early as bud burst and flowering time. According to previous studies, the temperature values recorded during these months are significant, as they provide critical insights into initiating the primary growth cycle and the timing of flowering events (Molitor et al., 2014; García de Cortázar-Atauri et al., 2017). These findings, especially for bud burst (EL 4) in 2024, support the understanding that a rapid growth period occurs in this location. High temperatures may shorten the grapevine growth cycle between bud burst (EL 4) and flowering (EL 26). The highest average maximum temperatures in May over the past six years were recorded in 2021. However, May 2020 experienced exceptionally high temperatures that adversely affected the berry set (EL 27) of cv. Sultana Seedless and cv. Sultan 7 grapes, with temperatures surpassing 40°C for five consecutive days during their flowering period (Teker and Soltekin, 2023). In contrast, May 2024 experienced notably lower temperatures compared to previous years. Upon reviewing the May temperature data across multiple years, it has become evident that the lowest mean temperatures were observed in May 2023 and May 2024. Flowering occurred earlier in 2024 than in previous years; however, low May temperatures following a warm April slowed down growth.

In the summer, the maximum average temperature was recorded as 38.01°C in June 2024. While temperature values from previous years were similar, July 2020 reached the maximum temperature at 38.43°C, and August 2023 experienced a peak of 40.33°C. In 2024, monthly average temperatures reached the highest levels for June, July, and August, with values recorded at 29.01°C, 29.99°C, and 29.24°C, respectively, compared to the average values between 2019 and 2023 (Figure 1F-H).

Analysis of minimum temperatures showed remarkable fluctuations between previous years and 2024 because, for January through April, the differences between 2019 and 2024 were mainly due to changes in the day distribution of minimum temperatures rather than daily averages (Figure 2A). The years with the highest number of days in January recording minimum temperatures below 0°C were 2020 (17 days) and 2022 (14 days). In contrast, February exhibited a notable increase in the number of days with temperatures dropping below 0°C, with 12 days recorded in both 2022 and 2023. In sharp contrast, 2024 experienced a significant decline, reporting only 2 days below 0°C (Figure 2B). March 2022 had a considerable number of days with temperatures below zero (10 days) and between 0°C and 5°C (21 days), characterizing it as a particularly cold month because there were no days with temperatures ranging from 5°C to 10°C or from 10°C to 20°C (Figure 3C). Furthermore, it was found that 2020 (16 and 4 days, respectively) and 2024 (14 and 4 days, respectively) had a similar number of cold days during these temperature intervals. Additionally, in April 2024, no recorded day was below 5 °C, and the number of days with temperatures between 10 and 20 °C (22 days) was determined to be higher than in previous years, 2019-2023 (Figure 2D).

As for maximum temperatures, national official records indicate that April 2024 was the hottest month in the past 53 years (TMS, 2024a). This study showed that many days during that month experienced temperatures in the ranges of 25°C to 30°C and 30°C to 35°C in 2024 (Figure 3A). However, in 2023 (24 days), as well as in 2019 (23 days) and 2021 (22 days), there was a significant number of days with temperatures ranging from 10°C to 25°C. In May 2024, data indicated that the number of days with high temperatures between 25°C and 30°C, as well as between 30°C and 35°C, was comparable to the number of such days recorded in May 2023. Additionally, in the past six years, it was determined that May temperatures, particularly in 2020, reached levels exceeding 40°C (Figure 3B).

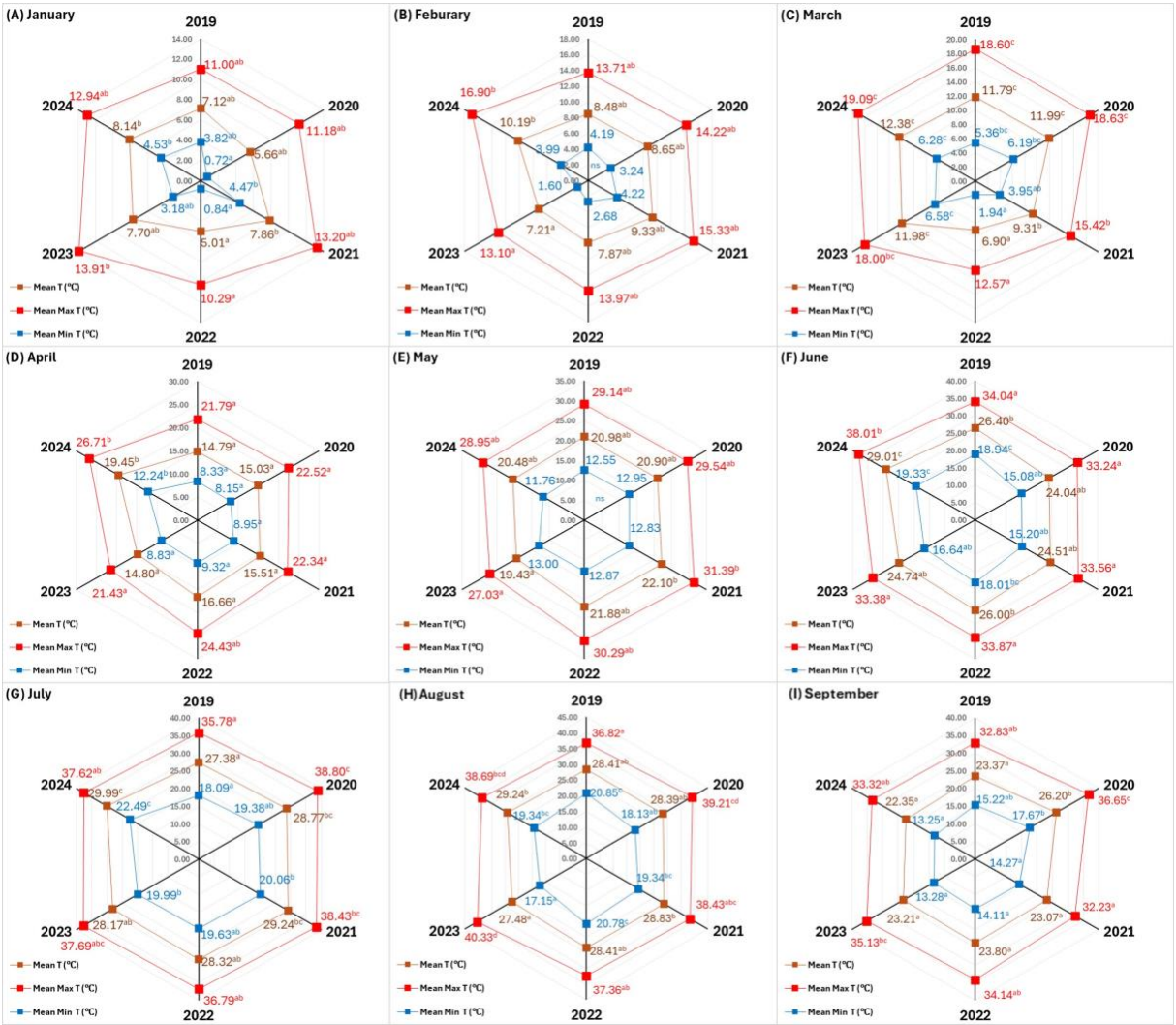

Figure 1. Monthly average minimum, mean, and maximum temperature data between January and September (A-I) from 2019 to 2024. Differences between years are indicated by letters of significance ($p \leq 0.05$). ns: non-significant.

During the summer months, particularly in June 2024, there was a significant increase in the number of days with temperatures ranging from 35°C to 40°C (24 days) exceeding 40°C (4 days). This trend made June markedly hotter in comparison to previous years. Additionally, this observation is substantiated by data indicating that temperatures in June reached unprecedented levels across the country, marking the highest recordings in the past 53 years (TMS, 2024b). A decrease in hot days was noted in July 2024; however, data showed that the highest temperatures exceeding 40°C were recorded in 2020 (11 days) and 2021 (9 days) (Figure 3E). Moreover, an analysis of temperature data reveals that August 2024 (7 days) experienced a lower frequency of high-temperature days than August 2023 (18 days), characterized by exceptionally intense heatwaves (Figure 3E).

Winkler Index values of the years

In April 2024, the WI value started with 283.41 GDD, indicating significantly warmer conditions compared to previous years, which recorded values ranging from 143.57 GDD to 199.66 GDD. As illustrated in Figure 1D, the average and maximum temperatures in April were higher than in previous years, further supporting this observation. Despite the low GDD value observed in previous years, except in 2023, a similar trend emerged in June when the GDD value reached 570.29. The maximum temperatures in July 2024 were comparable to those in previous years; however, a significant increase was observed in June due to elevated average and minimum temperatures (Figure 1G), contributing to a GDD of 620.28 (Figure 4). The WI value in 2024 was notably high due to the increased temperatures recorded in April and June during the growing season. The total WI for 2024 reached 2,945.01 GDD. By contrast, the year with the lowest GDD was 2023, with a total of 2,538.57 GDD (Figure 4).

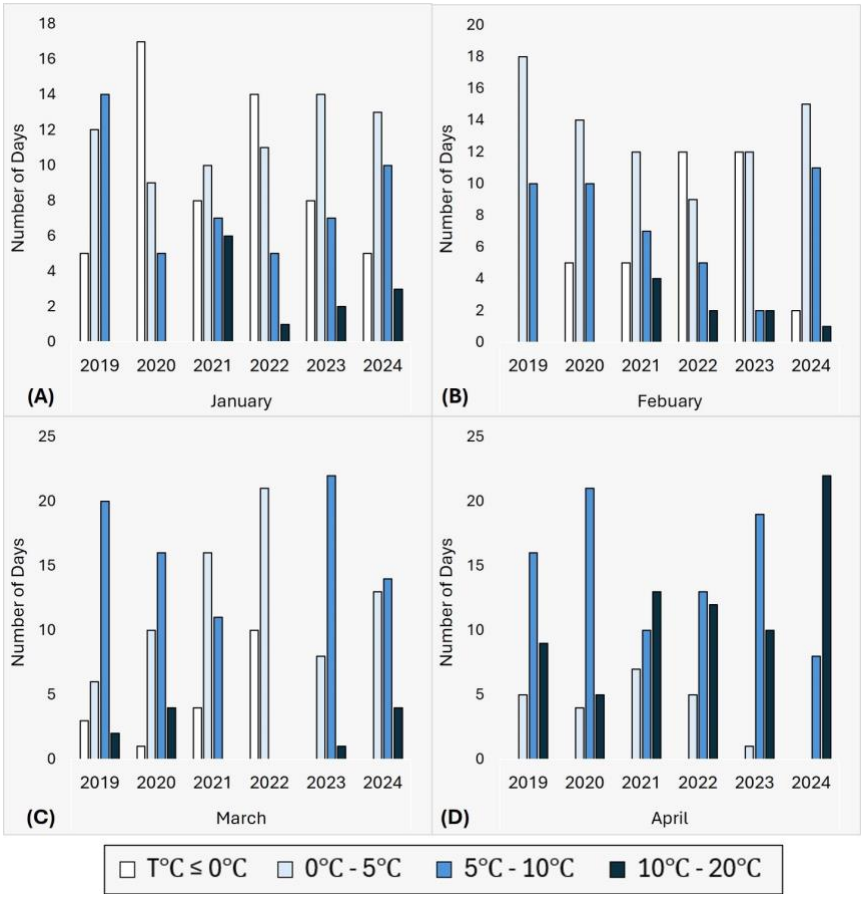

Figure 2. Illustration of the number of days with minimum temperatures in the ranges of T°C ≤ 0°C; 0°C - 5°C; 5°C - 10°C; and 10°C - 20°C from January to April (A-D) for the years 2019 to 2024.

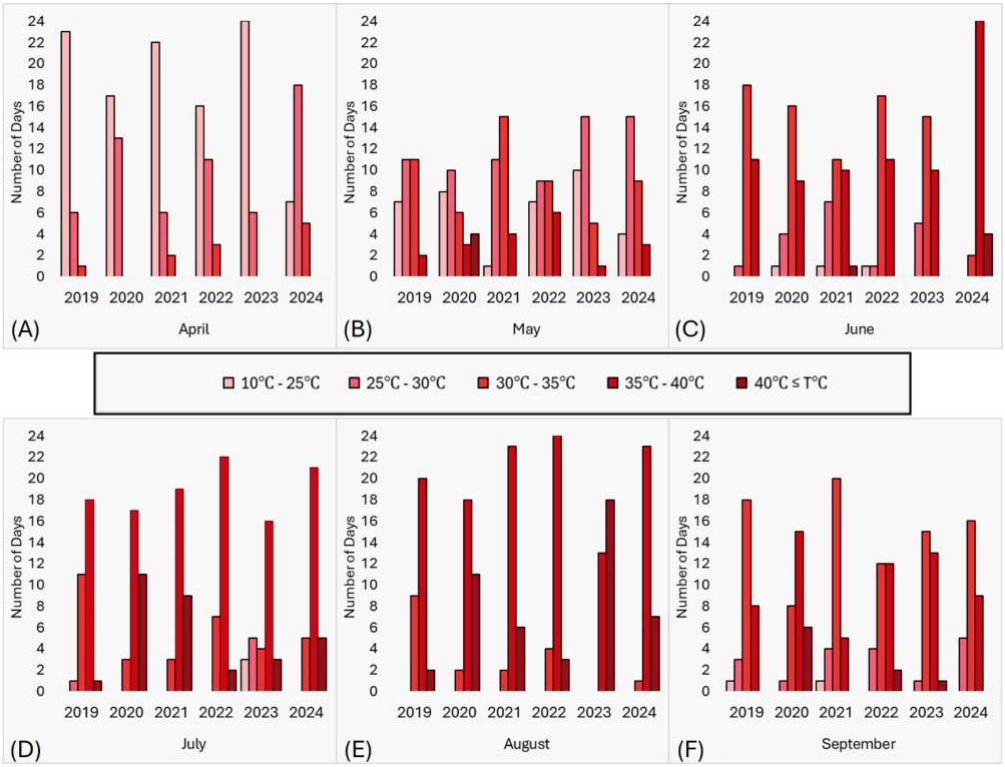

Figure 3. Illustration of the number of days with maximum temperatures in the ranges of 10°C - 25°C; 25°C - 30°C; 30°C - 35°; 35°C - 40°C and 40°C ≤ T°C from April to September (A-F) for the years 2019 to 2024.

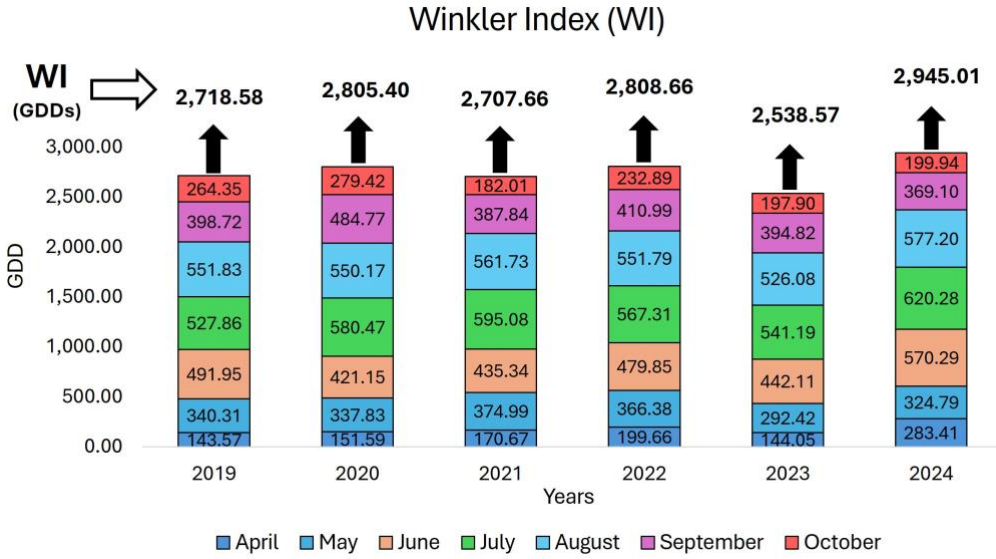

Figure 4. The graph of the calculation of Winkler Index values by months for 2019-2024.

Vegetation period and growing degree days

The period of grapevine phenological phases changes by season and is mainly impacted by climate (Fraga et al., 2012; Santos et al., 2020; Cameron et al., 2022). This study examined the variations in the length of the growth cycle of grapevines (Figure 4) and total effective heat summation values for the selected cultivar cv. Sultan 7 (Figure 5), providing a knowledge of yearly differences. The vegetation periods for grape variety were pretty much the same between 2019 and 2023. However, the vegetation period in 2024 was shorter than in 2019 and 2023 (Figure 4). Although the cultivar experienced its longest vegetation period in 2020 and 2023 (248 days), 2024 also experienced its shortest vegetation period (228 days).

There is a noticeable difference in bud burst and flowering timings in 2024 between 2019 and 2023. While bud burst varied between 79 and 85 days between the beginning of the year (January 1st) between 2019 and 2023, bud burst occurred in 75 days in 2024. Furthermore, while there was a lengthy difference between bud burst and full

bloom between 2019 and 2023, which varied between 51 and 67 days, the full bloom was completed on the 120th day of the year, 45 days after bud burst, as an early time interval in 2024. Cameron et al. (2022) found that the interval from budburst to flowering can be reduced more significantly than the intervals between berry set, veraison, and harvest, with a curvilinear relationship between these intervals and maximum temperature.

Previous research has investigated the interval between flowering and veraison. Malheiro et al. (2013) reported the strongest correlation between these two stages across all varieties studied. Additionally, Tomasi et al. (2011) noted that this interval exhibited variation, further supporting their conclusions.

This study determined that the interval between flowering and veraison ranged from 50 days in 2021 to 65 days in 2024. Remarkably, the difference between flowering and veraison was more significant in 2024, even though early flowering occurred that year. In contrast, the longest interval between veraison and harvest was 49 days, as seen in 2021 and 2023. The period between veraison and harvest also reduced to 41 days in 2022, 43 days in 2019, and 2024, and these years also experienced the shortest time from bud burst to full bloom (Figure 5). The variations in the durations during these periods have led to differing interpretations. Jones and Davis (2000) suggested that there could be a notable reduction in the intervals between flowering and veraison, flowering and harvest, veraison and harvest. Similarly, Bock et al. (2011) observed a trend of shortening in the interval between flowering and veraison in Franconia, Germany. Another study by Kartschall et al. (2015) reveals that in Germany, the acceleration of grapes' phenological development across all major stages may reach 11 ± 3 days, with harvest maturity occurring 13 ± 1 day earlier. Likewise, Duchêne et al. (2014) observed that in Alsace, France, phenological stages could advance by 8 to 11 days for budburst and by 16 to 24 days for ripening by the end of the 21st century. In contrast, Tomasi et al. (2011) did not identify a significant decrease in the intervals between flowering and veraison or between flowering and harvest. In addition, it is essential to note that the decisions made by viticulturists and winemakers can influence harvest timing (Tomasi et al., 2011; Cameron et al., 2022).

The analysis of all years in this study demonstrated that early flowering time (the day interval between bud burst and full bloom) may be more significant than the interval between flowering and veraison in predicting an early harvest date. Furthermore, it was noted that early flowering occurred in 2019, 2022 (DOY 136), and 2024 (DOY 120), all of which were associated with earlier harvest dates (DOY 240, DOY 236, and DOY 228, respectively).

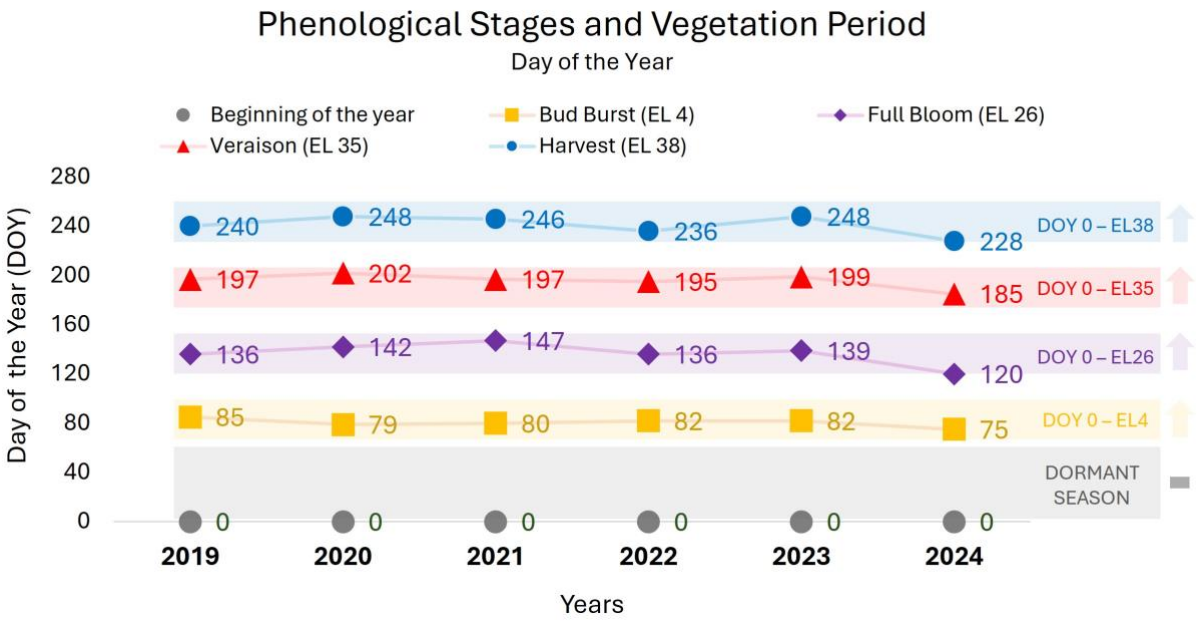

Figure 5. Illustration of the growing season length classified by phenological stages for 2019 to 2024. The grey line represents the entire dormant season; the yellow line indicates bud burst (EL4); the purple line shows full bloom (EL26); the red line marks veraison (EL35); and the blue line depicts harvest time (EL38), starting from the beginning of the year (DOY 0).

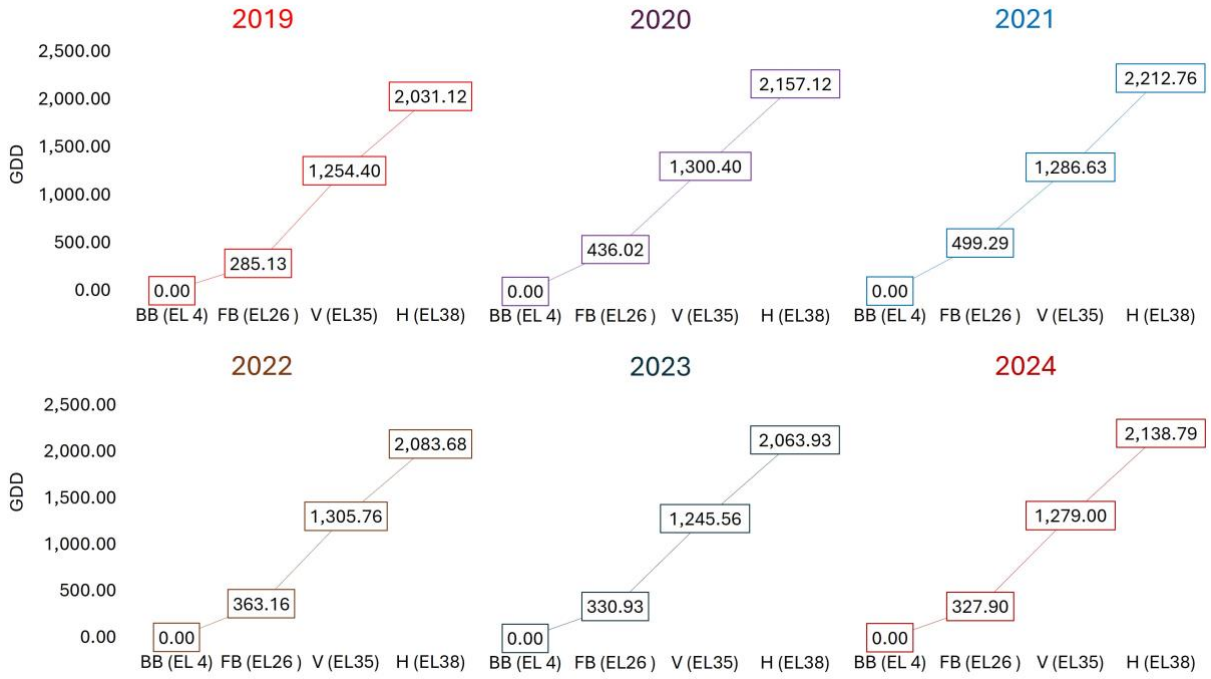

Figure 6. Illustration of the cumulative growing degree days classified by phenological stages of cv. Sultan 7 for 2019 to 2024. BB (EL4) represents the bud burst; FB indicates full bloom (EL26), V marks veraison (EL35), and H depicts harvest time (EL38).

The vegetation periods significantly influenced the effective heat summation of the variety each year (Figure 6). In 2020 (DOY 142) and 2021 (DOY 147), the late flowering times resulted in notably high effective temperature totals between bud burst and full bloom, with 436.02 GDD recorded in 2020 and 499.29 GDD in 2021. These figures were substantially higher than those observed in other years. Conversely, in the years 2019, 2023, and 2024, where flowering occurred earlier than in different years, it was observed that the GDD recorded between bud burst and full bloom were 285.13, 330.93, and 327.90, respectively. Notably, flowering in 2024 occurred on the 120th day, 16 days earlier than in 2019 and 2023. This early flowering was attributed to significantly high temperatures experienced before flowering in March and April. This study shows that the GDD value was higher than in 2019 in the same phenological phases in 2024, and the temperatures were higher in the same year (Figure 1). Therefore, the temperatures occurring before flowering in 2024 were relatively high compared to those in the last six years. On the other hand, despite a shorter vegetation period in 2024, it generated an effective heat summation value similar to 2020 and 2023. The study conducted by Templ et al. (2021) reveals a significant negative linear correlation (r = -0.62), indicating that as mean temperatures increase, the intervals within the DOY phenophase tend to shorten. This finding also underscores the impact of rising temperatures on phenological events.

CONCLUSION

Temperature distributions during the growing seasons in viticulture significantly influence the timing of phenological stages. Furthermore, variations in weather data across different years within a region can lead to changes in the monthly phenology of grapevines cultivated in that location. This study evaluates the monthly minimum, average, and maximum temperature values recorded over the past six years. It examines the Winkler Index and the changes in the vegetation period of the selected cultivar, cv. Sultan 7, and the distribution of these changes throughout the years and their respective phenological periods. Besides, the effective heat summation was calculated for the selected cultivar. As a result, the temperature data of the location in the last six years was higher in March, April, and June of 2024 compared to other years. In 2024, the flowering occurred earlier than in other years, and the harvest date was earlier than expected.

This study showed that an early bud burst and elevated temperatures in April led to a shorter interval between bud burst and flowering in the 2024 growing season. According to the Winkler Index calculation, the growing degree days value in April 2024 surpassed that of previous years between 2019 and 2023. In addition, June temperatures were unusually high, and July minimum temperatures were higher than those recorded in previous years. While the period (the number of days) between veraison and harvest has remained almost the same as in previous years, the harvest date occurred earlier than expected because both flowering and the beginning of veraison were determined earlier in the 2024 growing season.

Future studies should focus on short-term weather data in a specific unusual year, combined with comprehensive long-term research and potential climate scenarios, to enhance our understanding of recent changes in phenological stages due to elevated temperatures. These studies should examine the phenological phases of different grape cultivars over the coming years, particularly during warmer seasons and unusual weather events. The studies should also analyze these trends across various regions worldwide and compare them with previous seasons.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

TT designed the study and performed it. Wrote the paper and reviewed it.

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Reduction of gossypol in cottonseed meal using halophilic archaeal fermentation for enhanced feed safety and nutritional value

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Abstract

This study investigated the capacity of halophilic archaea fermentation to detoxify gossypol, a poisonous polyphenolic compound present in cottonseed meal, a major protein source in animal feed. The objective of the study was to investigate the ability of the fermentation process of using halophilic archaeal fermentation to decrease the level of gossypol in cottonseed meal as well as to increase the protein content of the diets. Halophilic microorganisms are able to survive in harsh environmental conditions, offer a potential answer for difficult industrial operations. Cottonseed meal contains high quantities of gossypol, was subjected to fermentation with the halophilic archaeon *Halorubrum ezzemoulense* in order to reduce gossypol levels and enhance protein content. The study entailed the fermentation of cottonseed meal with *H. ezzemoulense*, and the amounts of gossypol in the feeds before and after fermentation were assessed using HPLC. Results showed that there was significant ($p<0.05$) reduction (5.59±0.17 mg/kg) in the gossypol level after microbial fermentation compared to the control which was (139.03±7.17 mg/kg). The protein and lipid content of substrate increased significantly ($p<0.05$). These analyses revealed modifications in the nutritional values as a result of the fermentation process. The findings indicated a substantial decrease in gossypol levels, coupled with a remarkable rise in protein content. This novel technology not only tackled the drawbacks linked to cottonseed meal but also highlighted the capacity of halophilic archaea fermentation as a sustainable and efficient technique for enhancing the nutritional value of animal feed. Further research could focus on optimizing fermentation, exploring scale-up possibilities, and evaluating broader implications for the livestock industry.

Keywords: Cottonseed meal, Fermentation, Halophiles, Archaea, Gossypol

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INTRODUCTION

Cottonseed meal (CSM) is an important feed source in animal husbandry due to its high protein and other nutritional components. However, gossypol, a polyphenol pigment found in high concentrations in CSM and toxic to animals, limits the amount of CSM used in feed (Kaya et al., 1995; Umur et al., 2019). By inhibiting the activity of pepsinogen, pepsin and trypsin in the gastrointestinal tract, free gossypol reduces the digestibility of protein and binds iron in the diet (Devanaboyina et al., 2007). The poultry's reproductive performance was compromised when the diet contained an excessive amount of free gossypol (Zeng and Peng, 2013). The variation in poultry tolerance to cottonseed meal, the age and species of broilers. For this reason, many studies have aimed at reducing the amount of free gossypol in CSM through fermentation and to improve the nutrient profile as another output of this process (Zhang et al., 2006; Zhang et al., 2007).

Halophilic microorganisms are microorganisms that can live in salty environments. For this reason, there is no risk of contamination by other microorganisms in processes where halophilic microorganisms are used. Since

halophilic microorganisms are microorganisms that can withstand extreme conditions such as high salinity, temperature and UV, their application areas in industrial processes are also quite wide (Kesbiç et al., 2023).

Several studies has shown that the amount of free gossypol in CSM subjected to fermentation with different microorganisms decreases and especially the protein ratio increases (Sun et al., 2015). During fermentation, microorganisms utilize gossypol as a carbon source, metabolizing it into less toxic compounds while enhancing the nutritional value of the substrate (Zhang et al., 2006; Zhang et al., 2007). Contamination is one of the most important risks that can negatively affect the fermentation process. Contamination of feed plants by unwanted microorganisms during the fermentation process means that the process is not carried out correctly and as a result, the product is spoiled (Queiroz et al. 2018). In studies on the fermentation of CSM, bacteria (Wang et al., 2012), fungi (Zhang et al., 2006) and yeasts (Zhang et al., 2007) were used in the fermentation process. In order to prevent contamination and to perform fully controlled microbial management, the substrate is autoclaved before microorganism inoculation in CSM fermentation studies (Zhang et al., 2007), but this process means high fixed investment and operating costs in industrial applications. In this study, the microorganism used for fermentation is a halophilic microorganism which are known to possess specialized enzymatic pathways which could facilitate the breakdown of gossypol (Le Borgne et al., 2008). The potential of using gossypol as a carbon source not only opens up new possibilities for its detoxification but also enhances the nutritional profile of CSM, making it a viable feed source. Given the high salinity environments where halophilic microorganisms thrive, these strains could offer a unique solution to reducing contamination risks and eliminating the need for autoclaving in fermentation processes, providing both economic and practical benefits for large-scale applications. In addition, no published study was found in the literature search on the fermentation of CSM in halophilic microorganisms. The study is original in this respect and is thought to be both a source of inspiration for new studies and an incentive for industrial applications.

MATERIALS AND METHODS

Supply of Cottonseed Meal

The cottonseed was supplied from a local producer and cottonseed meal was obtained in a cold press oil machine in order to ensure that the meal did not undergo any processing.

Culture Conditions of Halophilic Archaea

In the study, a halophilic archaeon *Halorubrum ezzemoulense* DSM 19316 (Genbank accession number: AM048786) was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), which is stored at -20^{0}C in glycerol-added media, was used for fermentation. The microorganism was passaged into MAM JCM 168 medium and incubated at 39^{0}C for 3-7 days to have a fresh culture (Kesbiç and Gültepe, 2022).

Fermentation Process

A basal substrate containing cottonseed meal and wheat germ (3:2) was prepared. For the fermentation process, 100 g of substrate was inoculated with 10^8 concentration microorganism culture prepared with the salt-containing part of MAM JCM 168 medium (Trisodium citrate, $MgSO_4.7H_2O$, NaCl, KCl) in a way that it was moistened by 80 %. Fermentation continued at 39^{0}C for 21 days, and was kept at -20^{0}C to determine free gossypol level and the moisture, lipid, ash and protein percent of the product before fermentation and on the 21st day of fermentation.

Determination of Protein Amount of Cottonseed Meal

The Kjeldahl method was used to determine the protein amount of the meal (AOAC, 1975) and the analysis was performed in 3 replicates. Protein analysis were performed before and after the meal is subjected to fermentation and the change in the percentage protein amount of fermentation was calculated with the obtained data.

Determination of Free Gossypol Amount of Cottonseed Meal

The dried sample was weighed and extracted with acetonitrile (20 g/ 100 mL), according to the method of Ricci et al. (2015). The gossypol values of the cottonseed meal before and after fermentation and the effect of fermentation on gossypol were determined by High Performance Liquid Chromatography (HPLC, Shimadzu LC 20-A Prominence, Japan) with PDA detector (SPD-M20A) at 380 nm, and Agilent C18 (5 μm; 4.6 x 150 mm) column. The mobile phase A was acetonitrile (100 %), and mobile phase B was 0.3 % methanoic acid at the flow rate of 1.0 mL/min (Cheng et al., 2018).

Moisture Analysis

Before analysis, samples were weighed and placed in tared aluminum foil containers and dried in an oven at 50^{0}C until their weight remained constant.

Lipid Analysis

1 gram of dry sample was weighed for lipid analysis. After, the samples were kept in methanol-chloroform mixture in capped test tubes and filtered. They were taken into the first weighed flasks and lipid extraction was

done in the evaporator. Afterwards, the flasks placed in the desiccator were weighed after reaching a constant weight. The amount of crude oil was calculated using the following formula (Folch et al., 1957).

% Crude Oil Amount = Weight change of the volumetric flask (g) / Sample weight (g) x 100

Ash Analysis

For ash analysis, a homogeneous 0.5 g of sample was taken and placed in porcelain crucibles that have been previously tared. Then, the crucibles were burnt in the incineration furnace at 525^{0}C for 12 hours. The ash content of the samples were calculated according to the weight change of the crucibles according to the method of (AOAC, 1998). Formula is stated below:

% Raw Ash Content = Weight change of porcelain crucible (g) / Sample weight (g) x 100

Imaging by Scanning Electron Microscope

The strain was imaged by the scanning electron microscope (SEM, FEI, Quanta FEG 250, United States) before inoculating the substrate and after 21 day of fermentation on CSM (Kesbiç and Gültepe, 2022).

Statistical Analysis

Data was tested using one-way variance analysis with a statistical package program. Differences between values was tested using the Tukey multiple range test and a significant level of 0.05 was accepted as an indicator of the difference.

RESULTS AND DISCUSSION

Nutritional and gossypol content of experimental groups is presented in Table 1. According to the results, after 21 day fermentation with a halophilic archaeon *Halorubrum ezzemoulense* DSM 19316, the gossypol level of cottonseed meal were significantly decreased ($p<0.05$). While the gossypol level was 139.03±7.17 mg/kg in the control sample, it was reduced to 5.59±0.17 mg/kg after microbial fermentatiton. In addition, the protein and lipid content of CSM substrate were increased from 17.48±0.06 to 23.10±0.62 %, and from 10.68±0.16 to 13.65±0.26 %, respectively. The amount of ash in the fermentation group was found to be significantly higher than the control groups due to the salt content added for both sanitation and the growth of the target microorganism.

Table 1. Nutritional and gossypol content of experimental groups.

	Control	Storage control	Halophilic fermentation	p value
Protein (%)	17.48±0.06^b	15.18±0.05^c	23.10±0.62^a	<0.001
Lipid (%)	10.68±0.16^b	10.75±0.30^b	13.65±0.26^a	<0.001
Ash (%)	4.67±0.09^b	4.68±0.12^b	19.49±0.16^a	<0.001
Gossypol (mg/kg)	139.03±7.17^a	52.67±1.53^b	5.59±0.17^c	<0.001

Figure 1 shows the calibration curve of gossypol standards. Calibration points were prepared as 1, 5, 10, 20, 50 mg/kg. (r^2=0.999).

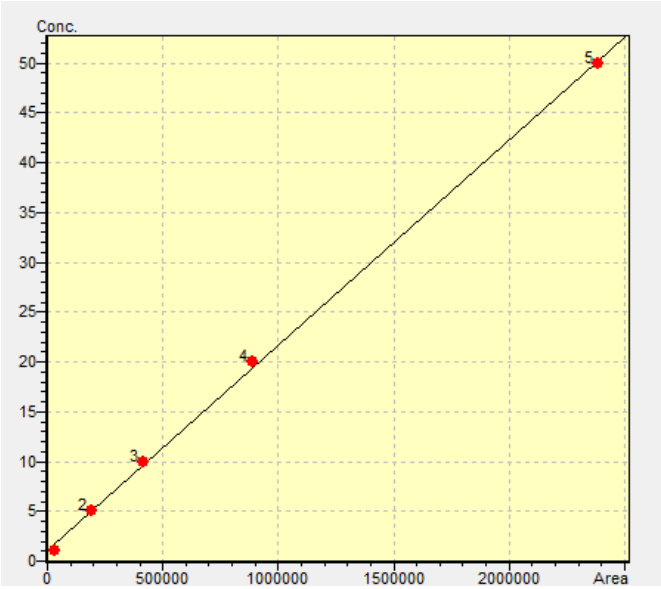

Figure 1. The linear calibration curve of gossypol standards.

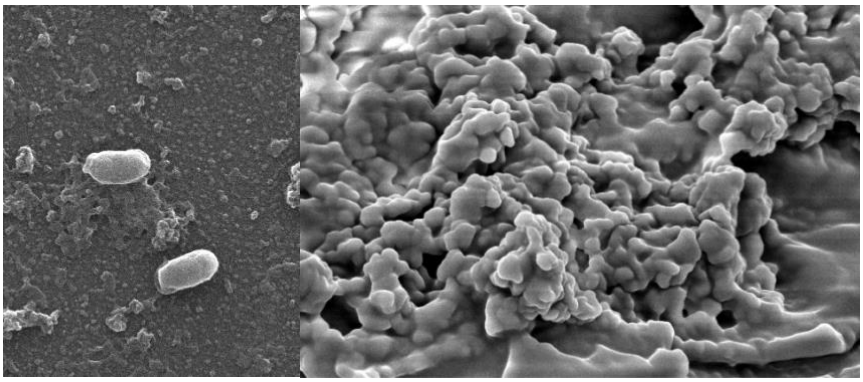

Figure 2. a) Images of *Halorubrum ezzemoulense*, b) after 21 day fermentation, *H. ezzemoulense* colonies on the CSM substrate.

The most important factor restricting the use of plant resources as a nutrient, in animal nutrition is the antinutrient contents of plants (Soetan and Oyewole, 2009). With the development of technology, the unwanted content of nutrients is being eliminated. However, disposal of the separated content adds additional costs to the process. For these reasons, fermentation is a long-standing method for conserving, improving and reducing antinutrient content of plant sources. (Jeyakumar and Lawrence, 2022).

The primary function of fermentation used as a feed technology is to extend the shelf life of the product and make it available for consumption. Its secondary importance is to improve the nutritional profile of the source. The most common use of the fermentation process in plant sources is silage production. In this process, wild fermentation is used, that is, the product is taken into a closed environment, an anaerobic atmosphere is provided and the plant is subjected to fermentation (Carvalho et al., 2014).

Another version of this process is the fermentation of plants with inoculants. In this case, the substrate should be sanitized to protect it from undesirable microorganisms. This leads to additional costs for the fermentation process. In our research, we tested the nutritional performance of silage of cottonseed meal, which is a common source of protein in livestock, using a halophilic archaea species. Our findings showed that gossypol, the most important antinutritional factor in cottonseeds, decreased by 96 % after 21 days of fermentation. This ratio is consistent with the boundaries of national feed declarations and international authorities in the field of animal feeding. According to the study of Zhang et al. (2006), fermentation of CSM with selected fungus species. The result of this study supports the findings of Zhang et al. (2006). This study achieved gossypol inhibition with a higher performance.

The plant's nutrient profile may change as a result of the fermentation of plant sources. During the fermentation process, microorganisms metabolize the vegetable source content and thus reproduce. As a result, a decrease in the content of cellulose, gossypol, etc. in the plant may result in a proportional increase in nutrient content, such as protein and fat. The findings of our current research support this statement. After 21 days of fermentation, an inverse ratio of gossypol and protein-fat content was observed in the cottonseed meal. Cottonseeds are among the most frequently used sources, in animal feeding. However, due to the toxicity caused by the gossypol content, it can be included in rations in a limited proportion. Therefore, cottonseed meal should be depurated before being used as a ration. The easiest method used in this cleansing process was to heat the substrate. In this case, the gossypol will deteriorate. However, as seen in our study, there are many advantages in purifying gossypol by microbial fermentation. Gossypol may decrease when the temperature is applied to the cottonseed meal, while denaturation-related protein may be decreased. In addition, heat-induced processes can cause the oil in the meal to oxidize. Xu et al. (2022) reported that heating decreased the protein in cottonseed meal (2022). Another study found that high-temperature treatment triggered fat oxidation in the meal (Waheed et al., 2004). In our study, the protein and fat ratio of the group fermented with *Halorubrum ezzemoulense* showed a significant ($p<0.05$) increase while the gossypol levels decreased.

This change is not believed to be due solely to the metabolism of the contents in the plant by the microorganism. It is known that plant resources, especially industrial waste, are used as a source of carbon and as a culture vessel to produce single-cell proteins. During fermentation, microorganisms produce proteins and lipids using carbon and nitrogen sources. This is an efficient way to support both the zero waste process and to generate added value from waste. As we did in our study, microorganisms reproduce using plant source content as a source of carbon. As a result, the protein and lipid content of the multiplied microorganisms contributed to the nutritional content in the total product. And the research we've done has some evidence that supports this

process. While the fat content of products stored at operating temperature did not change and the protein content showed reduction, fermentation group resulted in a significant ($p<0.05$) increase in these rates.

CONCLUSION

A halophilic archaeon, *H. ezzemoulense* is efficient microorganism for fermentation, which reduces the gossypol ratio of the cottonseed meal without requiring sanitation, and increases the protein-lipid ratio. Halophilic archaea are advantageous over other microorganisms because they can bypass the sanitation process in industrial applications. It is recommended to test the gossypol reduction and fermentation performance of different species in future.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

FIK designed the study and performed it. Wrote the paper and reviewed it.

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Effects of harvest time and plant part on essential oils, phenolics, and antioxidant activity in *Lippia citriodora*

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Abstract
This study investigates the effects of harvest time and plant parts on the concentrations of essential oils, phenolic content, flavonoids, and antioxidant activities (ABTS and DPPH) in the *Lippia citriodora* plant to determine the best outcome. The experiment consists of four different harvest times during flowering period and two plant parts (upper and lower). Harvest times were scheduled at weekly intervals.Significant variations were observed during experiment, Essential oil (EO) content reached peak value at the first harvest in upper parts of plants (L1U: 1.18%) and lowest value in lower parts by the fourth harvest (L4L: 0.25%). The highest phenolic content determined at the first harvest (L1: 44.04 mg GAE/g DW), while flavonoid levels reached peak value at the fourth harvest (L4: 314.07 mg rutin/g DW). Antioxidant activities, measured by ABTS and DPPH assays, were significantly greater in lower plant parts. Partial Least Squares Discriminant Analysis (PLS-DA) and Principal Component Analysis Discriminant Analysis (PCA-DA) confirmed clear distinctions between the upper and lower parts of the plant regarding bioactive compounds concentrations. The findings shows the importance of targeted harvest timing and plant parts in optimizing bioactive compound in *Lippia citriodora*, with implications for getting better benefits from plant.

Keywords: Plant parts, Harvest times, Bioactive compounds, Lippia

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INTRODUCTION

Lippia citriodora, or Lemon verbena, is a species of the Verbenaceae family, primarily cultivated because of its aromatic lemon-scented leaves, which are widely used in herbal teas. This herbal teas are believed to have antispasmodic, fever-reducing, sedative, and digestive benefits (Carrera-Quintanar et al., 2010; Lenoir et al., 2011). *L. citriodora* serves both culinary and medicinal purposes, being abundant in flavonoids and phenolic acids, which enhance its therapeutic properties. Moreover, its essential oil is extensively utilized in pharmaceuticals, perfumes, and cosmetics (Kaskoos, 2019). *L. citriodora*, originally from South America, especially Argentina, Brazil, and Paraguay, has been extensively cultivated in southern Europe and North Africa for its medicinal and aromatic properties (Aghdam et al.,2019; Farahmandfar et al., 2018). The plant grows in warm climates and is commonly cultivated in gardens for its aromatic leaves (Argyropoulou et al., 2010; Ebadi et al., 2016; Kara et al., 2018).

Lippia plants also serve as important sources of essential oils, which have been researched for their potential applications in the food industry (Mustafa, 2015; Kamal et al., 2011). The essential oils and bioactive substances found in medicinal and aromatic plants exhibit considerable variation influenced by internal and external factors, such as the specific plant part, growth stage, and harvesting time (Figueiredo et al., 2008; Telci et al., 2009).

The correlation among harvesting time, plant parts, and the chemical composition of medicinal plants is crucial for optimizing their therapeutic efficacy. This is particularly important for tall plants such as *Lippia citriodora*, which can attain heights of 3 to 5 meters. The upper regions of the plant are exposed to much more sunlight than the lower areas, which may cause variations in the concentration of bioactive compounds. Understanding these variations are important for enhancing the plant's medicinal and culinary efficacy. This study aims to assess the

variations in bioactive content across different harvest times and plant parts to maximize the potential benefits derived from the *Lippia citriodora* plant.

MATERIALS AND METHODS

Plant Material

The plant material used for the current study was *Lippia citriodora*, cultivated at the Faculty of Agriculture, Aydın Adnan Menderes University. Plant material were six years old, fully grown and mature. The harvests were done during the fall, blooming period (23 October-13 November), ensuring the plants were at a suitable stage of development for the extraction of bioactive chemicals. Harvest proceeded in several phases, with a weekly gap between every harvest. This approach allowed us to observe variations in chemical composition that could occur throughout the period of flowering.

Experimental Design

The experiment consists of four different harvest times (L1, L2, L3, and L4) and two distinct plant parts.Harvest times were spaced weekly. The plant parts were divided into two sections: the upper and lower portions of the plant. To avoid the effects of diurnal variability, all harvests were conducted between 10:00 AM and 12:00 PM. After harvesting, the plant samples were shade-dried to minimize the loss of bioactive substances.The samples were taken in four replicates, and the experiment was designed according to a completely randomized factorial design. The plants left to dry in the shade were weighed daily until their weight stabilized, indicating that they were fully dried. Once dried, the stems and the drog (dried medicinal plant parts used for medicinal or culinary purposes) parts were separated, and the following analyses were performed on drog parts of plant.

Determination of essential oil content

The essential oil content was assessed using the hydro-distillation method. Ten grams of dried plant material were added to 100 mL of distilled water and hydrodistilled using a Clevenger-type equipment. The distillation process took one hour at a temperature of 180°C. After the distillation process was finished, the mixture was allowed to cool for 5 minutes so that any condensed vapor may settle. The essential oil was then accurately quantified and collected. The oil content (%) was calculated using the following formula and expressed as a percentage.

Oil Content (%) = (Dry Sample Weight (g) / Extracted Oil Volume (ml)) × 100

Extraction of samples

Extraction was done on dried plant samples following the method of Skerget et al., (2005), with slight modifications. The samples were ground and subsequently sieved to ensure uniformity. A 500 mg portion of the dried powdered sample was mixed with 50 ml of 80% methanol and extracted in a shaking incubator at 40°C for 2 hours (150 rpm). Then, the extract was filtered and immediately used for the analyses described at below.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The evaluation of antioxidant capacity was carried out via the DPPH assay, following to the methodologies defined by (Gadow et al., 1997; Maisuthisakul et al., 2007). A portion of 100 µL of the extract was diluted to create four different concentrations, afterward added to 3.9 mL of a freshly prepared 0.1 mM DPPH solution (2,2-diphenyl-1-picrylhydrazyl radical). The mixture was gently shaken and then allowed to incubate in the dark at room temperature (23-25°C) for a duration of 30 minutes. Following the incubation period, the absorbance of the final solution was assessed spectrophotometrically at 516 nm with the aid of a microplate reader. The antioxidant activity of the samples was represented as Trolox equivalent antioxidant capacity (mg TEAC/g DW).

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay

The ABTS assay was performed according to the method described by Re et al., 1999. To generate the ABTS radical cation (ABTS•+), a 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate (1:1 ratio) and allowed to react in the dark for 16 hours. The resulting ABTS•+ solution was then diluted with methanol to achieve an absorbance of 0.700 at 734 nm. A 5 µL aliquot of plant extract was added to 3.995 mL of the diluted ABTS•+ solution and incubated for 30 minutes in dark. After that the absorbance of sample was measured, and the antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (mg TEAC/ g DW).

Determination of total flavonoid content

To determine the flavonoid content, 0.5 mL of the sample extract was combined with 2.5 mL of distilled water and 150 µL of 5% sodium nitrite (NaNO2), followed by gentle shaking. The mixture was left to stand for 5 minutes, after 300 µL of 10% aluminum chloride ($AlCl_3$) was added and allowed to react for another 5 minutes. Subsequently, 1 mL of 1 M sodium hydroxide (NaOH) was added, and the total volume was adjusted to 5 mL with distilled water. The solution was then incubated for 30 minutes, and the absorbance was measured at 510 nm using a microplate reader. The flavonoid content was expressed as rutin trihydrate equivalents (MW: 664.56), following the method described by Chang et al., 2006.

Determination of total phenolic content

The phenolic content was determined using the procedure described by Skerget et al., 2005. To perform this procedure, mix 0.5 mL of sample extract, 2.5 mL of 0.1 N Folin-Ciocalteu reagent, and 2 mL of sodium carbonate solution (75 g/L Na_2CO_3). The reaction mixture was incubated for 5 minutes at 50°C to promote the formation of

the blue color, which shows the presence of phenolic chemicals. Following incubation, the mixture was shoked with ice and cooled to room temperature. The absorbance of the solution was then measured at 760 nm with a microplate reader. The phenolic content was represented in gallic acid equivalents (mg GAE per gram dry weight).

Statistical Analyses

The data were subjected to a one-way analysis of variance (ANOVA) to evaluate differences among treatment groups. Following ANOVA, multiple comparisons between treatment levels and controls were performed using Tukey's HSD, post hoc test to control for multiple comparisons. Additionally, Principal Component Analysis (PCA), incorporating discriminant analysis to enhance group separation, and Partial Least Squares Discriminant Analysis (PLS-DA) were conducted to further explore patterns and relationships within the data. All statistical analyses were conducted using JMP Pro 16 software (SAS Institute, Cary, NC, USA), while data visualization, including the PCA-DA and PLS-DA plots, was carried out using Python's Matplotlib library.

RESULTS AND DISCUSSION

ANOVA results

Table 1 shows the ANOVA results for essential oil content (EO), phenolic content (Phn), ABTS and DPPH radical scavenging capabilities, and flavonoid content (Flv). Harvest time (HT), plant part (PP), and their interaction (HT x PP) had a significant impact on all evaluated parameters, as indicated by the P values. Harvest time showed highly significant effects ($P<0.01$) across all parameters. Plant part also had significant effects, with phenolic content significant at $P<0.05$ and other parameters at $P<0.01$. The interaction between harvest time and plant part (HT x PP) was significant for all parameters at $P<0.01$.

Table 1. Effects of Harvest Time (HT) and Plant Part (PP) on Essential Oil (EO), Phenolic Content (Phn), ABTS Radical Scavenging Activity (ABTS), DPPH Radical Scavenging Activity (DPPH), and Flavonoid Content (Flv) in *Lippia citriodora* Plants.

Factors		**EO**	**Phn**	**ABTS**	**DPPH**	**Flv**
				HT (harvest time)		
L1		0.85 **ab**	44.04 **a**	101.14 **a**	104.74 **a**	239.95 **c**
L2		0.95 **a**	33.59 **c**	70.87 **c**	78.02 **d**	262.89 **b**
L3		0.73 **bc**	35.81 **c**	85.67 **b**	83.37 **c**	226.36 **d**
L4		0.63 **c**	40.67 **b**	85.58 **b**	92.63 **b**	314.07 **a**
				PP (plant part)		
L		0.62 **b**	37.68 **b**	70.26 **b**	86.78 **b**	246.33 **b**
U		0.95 **a**	39.37 a	101.37 **a**	92.60 **a**	275.30 **a**
				HTxPP		
L1	L	0.52 **d**	40.74 **b**	69.91 **e**	104.19 **a**	229.00 **f**
L2	L	1.02 **ab**	33.88 **d**	63.31 **e**	70.83 **d**	284.40 **b**
L3	L	0.70 **cd**	36.69 **bcd**	100.78 **c**	84.35 **c**	190.71 **f**
L4	L	0.25 **e**	39.42 **bc**	47.03 **f**	87.75 **c**	281.22 **b**
L1	U	1.18 **a**	47.35 **a**	132.37 **a**	105.28 **a**	250.91 **cd**
L2	U	0.88 **bc**	33.30 **d**	78.43 **d**	85.21 **c**	241.37 **de**
L3	U	0.75 **cd**	34.93 **cd**	70.56 **e**	82.40 **c**	262.00 **c**
L4	U	1.00 **ab**	41.91 **b**	124.13 **b**	97.52 **b**	346.93 **a**
Anova	df			***Mean Square***		
HT	3	0.124**	133.532**	916.579**	822.392**	8927.054**
PP	1	0.657**	17.135*	5808.947**	203.558**	5035.829**
HT*PP	3	0.293**	20.951**	3557.762**	85.801**	4186.839**

*EO: Essential Oil, Phn: Phenolic Content, ABTS: ABTS Radical Scavenging Activity ,DPPH: DPPH Radical Scavenging Activity, Flv: Flavonoid Content, HT: Harvest Time ,PP: Plant Part (HTxPP: Interaction between Harvest Time and Plant Part df: Degrees of Freedom and four harvest times (L1, L2, L3, L4)

A significant reduction in essential oil content (EO) was observed in the lower parts of the plant during all harvests, particularly in the fourth harvest (L4L: 0.25%). The harvest time significantly influenced the essential oil content showing a consistent pattern of increased in the early harvests (L1 and L2) and a decline in following harvests (L3 and L4), particularly in the lower parts of the plants. The upper parts (U) consistently exhibited a greater essential oil content than the bottom parts (L) during all harvests. The essential oil amount in the upper parts remained comparatively elevated in successive harvests (L4U: 1.00%), whereas in the lower parts, the essential oil content significantly decreased over time (Figure 1).

The results showed a significant variation in EO content at various harvest times. The second harvest recorded the highest concentration (L2: 0.95%), whereas the fourth harvest showed the lowest concentration (L4: 0.63%). The observed variations could result from physiological shifts occurring during the plant's growth cycle, which may impact the synthesis of secondary metabolites (Verma et al., 2015). The upper parts of the plants consistently demonstrated a higher essential oil content than the lower parts (U: 0.95%, L: 0.62%). It is known that environmental factors, including temperature, humidity, and soil nutrients, significantly influence essential oil production. The consistent trend observed in the upper parts of plants indicates that light availability can be a primary factor in here. Research by Zhao et al. (2022) shows that the synthesis of essential oils is closely associated with the metabolic pathways activated during photosynthesis. The upper leaves, due to their greater contact with light, show higher chlorophyll content, enhancing photosynthetic efficiency and resulting in increased essential oil production. Research conducted by Malayeri et al. (2010) shows that changes in light intensity can significantly affect both the composition and the quantity of essential oils produced. The relationship between harvest time and plant part suggests that harvest timing matters for optimizing essential oil production, as the highest essential oil content was recorded in the upper part of the plant during the early harvests during flowering period. The studies by Karık et al. (2019) and Toncer et al. (2022) on *Lippia citriodora* shows slightly different results, likely due to variations in methodology. Both studies focus on a single harvest, which may explain the discrepancies. For instance, Toncer et al. (2022) reported significantly lower essential oil content (0.32%-0.37%) with lower parts have higher values than upper parts of plants, while Karık et al. (2019) found higher essential oil content in the upper parts of the plant in (10 a.m.) morning harvest conditions but they obtained the opposite results under (4 p.m.) afternoon conditions. These differences in findings lead to varying conclusions and interpretations. In our study, the highest average essential oil content across all harvest times was obtained from the upper parts of plant. However, when examining individual harvests, it was observed in L2 harvest that lower parts of plant exhibit higher essential oil content compared to the upper parts of plant which similiar with Karık et al. (2019) and Toncer et al. (2022). This indicates that, although the essential oil content may vary between different plant parts depending on the harvest period, on average, the upper parts of plants contain higher levels of essential oil than lower parts of plants.

Phenolic content varied significantly across different harvest times, the highest level of phenolic content recorded during the first harvest (L1: 44.04 mg GAE/DW) and the minimum levels observed during the second and third harvests (L2: 33.59 mg GAE/DW, L3: 35.81 mg GAE/DW). The phenolic content (Phn) exhibited a small rise when it comes to the fourth harvest (L4L: 39.42 mg GAE/DW, L4U: 41.91 mg GAE/DW); however, these values remained lower compared the first harvest. The upper plant parts showed a consistently increased phenolic content compared to the lower parts across all harvests, significant difference between the lower and upper parts was observed during early harvesting times, with the upper parts showed higher phenolic content. The interaction between harvest time and plant part demonstrates that accurate timing of harvest is important for improving both essential oil and phenolic production, especially as the upper part exhibited the highest phenolic concentration during the first harvest (L1U: 47.35 g GAE/mg DW). When evaluating the ABTS value, the second harvest exhibited the lowest overall values, particularly in the lower plant parts (L2L: 63.31 mg/g DW), indicating a reduction in ABTS activity during this period. Similar to phenolic content, the upper plant parts consistently demonstrated higher ABTS activity compared to the lower parts across all harvests. This difference can be seen especially in both the first and final harvests. ABTS activity in the lower plant parts was generally below the overall mean, except during the third harvest (L3L: 100.78 mg/g DW). The DPPH activity was lowest in the second harvest (L2), in the lower portion of the plant (L2L: 70.83 mg/g DW). The fourth harvest showed an elevation in DPPH activity in the upper part of the plant (L4U: 97.52 mg/g DW). In the early harvest, DPPH activity in both the upper and lower sections was comparable, with both values above the overall mean, shows that the time of the first harvest produces the most balanced and strongest antioxidant activity throughout plant parts. In following harvests (L2, L3, L4), the upper sections continuously exhibited elevated DPPH activity relative to the lower sections, especially during the second and fourth harvests. DPPH activity maximized at the first harvest for both the upper and lower sections of the plant, but showed a considerable decline in the subsequent second and third harvests. The fourth harvest demonstrated a rise in DPPH activity, especially in the upper parts. The ABTS and DPPH radical scavenging activities reached its peak at the beginning of harvest, showing significantly higher activity in the upper parts of the plant. The relationship between antioxidant capacity and phenolic content suggests that phenolics are key contributors to antioxidant activity in *Lippia* (Feduraev et al., 2019). This finding shows the

upper parts of plants as an important source of natural antioxidants, with harvest timing also important. Gathering of samples during the beginning of harvest improves antioxidant activity.

The lowest flavonoid content was determined in the lower part of the plant during the third harvest (L3L: 190.71 mg rutin/g DW), but the higher part of the plant in the same harvest (L3U: 262.00 mg rutin/g DW) showed higher levels. The fourth harvest (L4) yielded the highest flavonoid content, while the third harvest showed the lowest values. The upper parts of the plant exhibited elevated flavonoid levels compared to the lower parts across all harvests, with the most significant differences observed during the third and fourth harvests. The maximum flavonoid concentration was observed in the upper part of the fourth harvest (L4U: 346.93 mg rutin/g DW), while the minimum content was found in the lower section of the third harvest (L3L: 190.71 mg rutin/g DW). The flavonoid concentration generally increased over time, reaching its peak around the fourth harvest. The upper parts consistently exhibited higher flavonoid concentrations compared to the lower parts in every harvest time. The flavonoid content was highest at the fourth harvest, with upper parts showing generally higher flavonoid levels than lower parts (L4: 314.07 mg rutin/g DW; U: 275.30 mg rutin/g DW vs. L: 246.33 mg rutin/g DW). The delayed maximum in flavonoid accumulation shows that flavonoids may be synthesized later in the flowering cycle. The raised flavonoid content in the upper plant parts at the fourth harvest (L4U: 346.93 mg rutin/g DW) likely shows improved synthesis due to extended sunlight exposure, which is recognized to affect flavonoid biosynthesis via light-responsive pathways (Wang et al., 2022). Zoratti et. al., (2014) and Shi et. al., (2024) also suggest that sunlight is a significant environmental factor inducing flavonoid biosynthesis and light exposure enhances flavonoid accumulation.

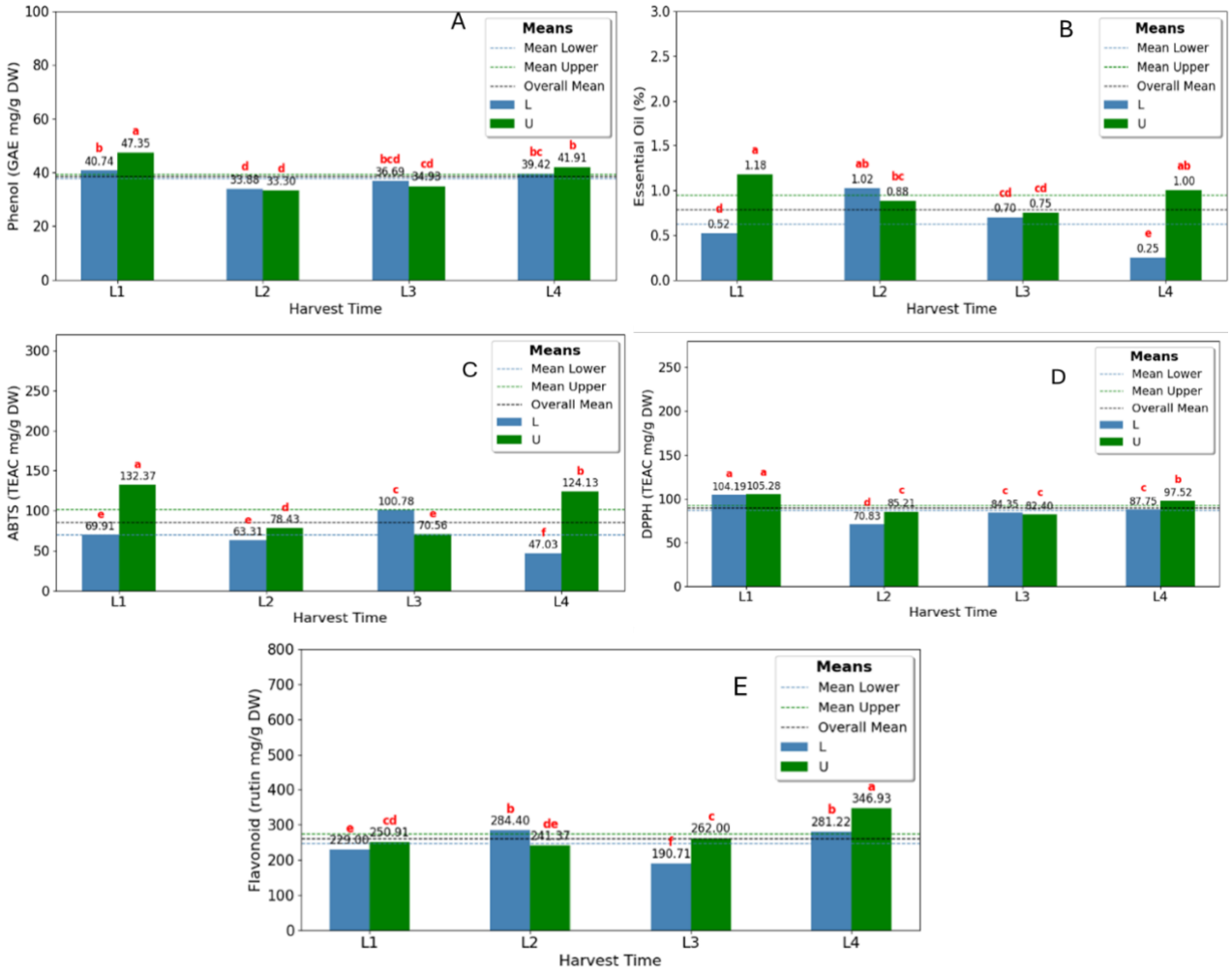

Figure 1. Variation in Phenol (A), Essential Oil Content (B), ABTS (C), DPPH (D) and Flavanoid (E) Between Lower (L) and Upper (U) Parts of the Plant Across Four Harvest Times (L1, L2, L3, L4), Highlighting the Interaction Between Harvest Time and Plant Part. (P<0.01).

PCA-DA and PLS-DA Analysis results

Partial Least Squares-Discriminant Analysis (PLS-DA) and Principal Component Analysis-Discriminant Analysis (PCA-DA) was used to further examine the results and determine variation in

bioactive parameters including flavonoids, essential oil (EO), ABTS, phenols, and DPPH, in different parts (upper and lower) of the *Lippia* plant across four separate harvest times.

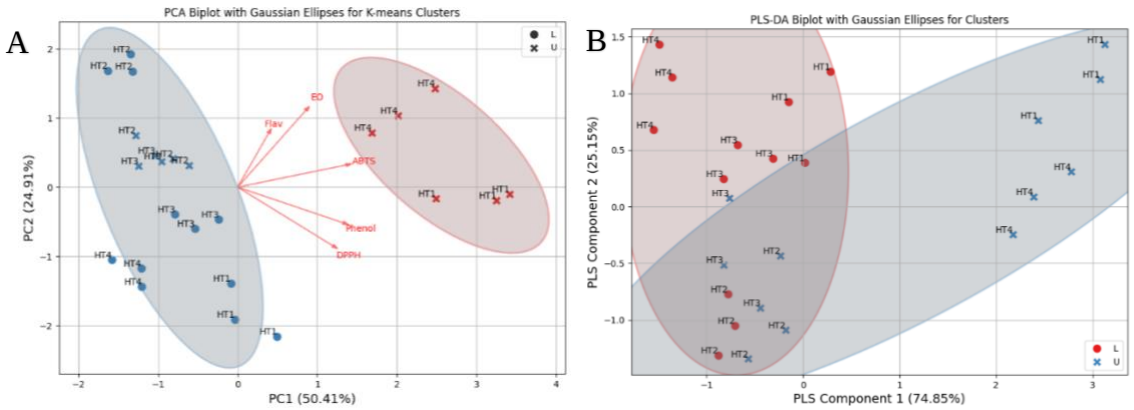

Figure 2. PCA-DA (A) and PLS-DA (B) biplots show the distinction of lower and upper plant parts according to specified parameters.

In the PCA-DA graph, the x-axis (PC1) accounts for 50.41% of the variance, while the y-axis (PC2) explains for 24.91%. Together, these two components reflect 75.32% of the overall variance in the data, the first two principal components capture an important portion of the variation (Figure 2A). The PCA graph illustrates two separate clusters represented by Gaussian ellipses: the blue ellipse on the left reflects samples assigned "HT1," "HT2," "HT3," and "HT4" within the "L" group (marked by filled blue circles), while the red ellipse refers to "HT1," "HT4," etc., classified as "U" (identified by red crosses). The two clusters clearly distinguish the two groups, linked to plant parts (L = lower, U = upper). The red arrows show the measured parameters. The position and size of the arrows indicate the effect of these parameters on the main components. Essential oil (EO), flavonoids (Flv), ABTS, phenol, and DPPH are factors affecting the differentiation of the clusters. Samples in the upper part (U) class show more impact from higher values of EO, Flav, ABTS, phenol, and DPPH compared to lower part (L) class. The L class (blue cluster) is positioned further from these variables, showing decreased values of these components relative to the U group.

PLS-DA is a supervised multivariate statistical approach that distinguishing the examimined parameters of the upper (U) and lower (L) parts of the plant and harvest times. The x-axis of the PLS-DA model (PLS Component 1) elucidates 74.85% of the variance, while the y-axis (PLS Component 2) reflects 25.15%. Together, both of these variables explain approximately 100% of the variance in the data, signifying that the PLS-DA model effectively captures almost all the information required for group distinction (Figure 2B).

The distinct separation within the two groups, shown with the red and blue ellipses, shows the efficacy of PLS-DA based on the defined parameters. The U category (upper parts of plants) is mainly located on the right side of the plot, while the L group (lower plant parts) positioned on the left. The observed separation proves that the variables used in this model are competent in distinguishing between the two groups. In contrast to the PCA-DA which shows greater overlap, the separation observed in PLS-DA is stronger, showing the supervised characteristic of PLS-DA.

CONCLUSION

This study shows the significant variation in the bioactive content of *Lippia citriodora*, influenced by both harvest timing and plant parts. Essential oil (EO) content peaked during the second harvest, with consistently higher levels observed in the upper plant parts across all harvests. This results may result from greater sunlight exposure in the upper regions compared to the lower parts. Similar patterns were observed for other parameters. Phenolic content reached its maximum during the early harvests, and antioxidant activities (ABTS and DPPH assays) followed a same pattern, exhibiting higher activity in the upper plant parts. Flavonoid content, however, peaked during the fourth harvest, dominantly in the upper sections of the plants. These findings underscore the importance of optimizing harvest timing during the flowering period and considering specific plant regions to maximize the yields of essential oils, phenolics, flavonoids, and antioxidants in *Lippia citriodora*. This can be used as practical implications for medicinal and culinary purposes of *Lippia citriodora* plant.

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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Prevalence of plant parasitic nematodes in irrigation water and soil in clove and tomato greenhouses in Isparta Province of Türkiye

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Abstract

The study was carried out to determine the contamination status of irrigation water with plant parasitic nematodes and the effect of their spread in the greenhouse soil. For this purpose, soil samples and water samples from the irrigation source of the greenhouse were taken from the same greenhouses in May and October in Isparta Province of Türkiye. A total of 20 samplings were collected from 13 tomato and 7 clove greenhouses. The irrigation sources of these greenhouses were notedas 8 wells and 12 open pools. Nematode densities in 100 g of soil and 1 l of water were determined. In the study, 8 economically important plant parasitic nematode genera (*Meloidogyne* spp., *Criconemoides* spp., *Helicotylenchus* spp., *Ditylenchus* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Xiphinema* spp. and *Tylenchus* spp.) were detected in irrigation water and soil samples. The percentages of presence of *Criconemoides* spp., *Helicotylenchus* spp., *Ditylenchus* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Xiphinema* spp. and *Tylenchus* spp. in soil were found to be 15%, 35%, 25%, 45%, 25%, 25% and 45%, respectively. The percentages of their presence in water samples were determined as 25%, 35%, 35%, 25%, 25%, 30% and 30%, respectively. In seven soil samples (S1, S6, S7, S13, S17, S20) *Meloidogyne* spp. has been found. Five of these samples (S6, S7, S12, S17, S20) belong to tomato greenhouses irrigated with pool water. While the S13 sample belongs to the clove greenhouse soil irrigated with pool water, the S1 sample was taken from the tomato greenhouse irrigated with well water. *Meloidogyne* spp. were in both soil and water samples of S1, S6, S7, S12, S13, S17 and S20. While S9 and S18 were only found in water samples. It appears that the likelihood of root knot nematodes being present is higher in greenhouses irrigated from open pools. In general, nematode densities were found to be higher in soil and water samples in October. While *Meloidogyne* spp. densities varied between 100-900 individuals/100 g of soil, they varied between 200-1400 individuals/1 L of water samples. In the study, significant evidence was obtained regarding the transmission of plant parasitic nematodes to greenhouse soil through irrigation water.

Keywords: Contamination, *Meloidogyne* spp., Plant parasitic nematode, Pool, Irrigation water

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INTRODUCTION

Isparta Province has a surface area of 893,307 ha. The size of agricultural lands is 210,078 ha. Under cover cultivation is practiced in 5,363 da area in Isparta Province. The most greenhouse cultivation areas are in the Center (2,565 da) and Yalvaç (1,612 da) districts in Isparta. Deregümü village is located in the Central District of Isparta Province. People of Deregümü village make their living from cloves and tomatoes in greenhouses. In the village where the number of greenhouses is gradually increasing, cloves grown on 1,850 decares of land are exported to Spain, Bulgaria, England, Holland and Russia after being stored in cold storage. Approximately 250 million branches of cloves are produced every year. Tomatoes produced on 750 decares of land in Deregümü also have

significant place in the domestic and foreign markets. Approximately 10 thousand tons of tomatoes are produced from 750 decares of land. More than half of the tomatoes are sent to provinces such as Ankara, Istanbul, Izmir and Konya, while some of them are exported (Anonymous, 2023, TUİK, 2023). Total water potential of Isparta Province are1,175 million m^3/year and groundwater potential is 91 million m3/year (DSI, 2016). Within 1, 068 993 hectares of agricultural land potential, the amount of land suitable for irrigation surveyed by DSI (State Hydraulic Works, Türkiye) is 574,532 hectares. A total of 408, 122 hectares of land is currently irrigated, of which 73, 599 hectares are underground irrigations and 334, 523 hectares are DSI operational and surface irrigations. There is an agricultural irrigation cooperative in Deregümü village. It is also observed that farmers drill water wells for agricultural irrigation. An area of 4,468 ha can be irrigated from wells licensed by DSI (Anonymous, 2023).

Plant parasitic nematodes are important pests that cause economically yield losses in cultivated crops worldwide. To date, more than 4,100 species of plant parasitic nematodes have been identified (Decraemer et al., 2006). However, not all of these species cause economic losses in plants. The economically important nematode genera or species are listed as *Meloidogyne* spp., *Heterodera* spp. and *Globodera* spp., P*ratylenchus* spp., *Radopholus similis*, *Ditylenchus dipsaci, Bursaphelenchus xylophilus, Rotylenchulus reniformis, Xiphinema index, Nacobbus aberrans* and *Aphelenchoides besseyi* (Jones et al., 2013; Devran and Mıstanoğlu, 2017). The majority of plant parasitic nematodes can cause damage the roots of their hosts, while very few of them can occur damage the above-ground parts of plants such as leaves, flowers or stems (Hunt et al., 2005). The damage caused by plant parasitic nematodes is estimated at US$ 80 billion per year (Nicol et al., 2011). The spread of plant parasitic nematodes from one field to another or from one region to another is caused by irrigation water, transportation of the soils in which they are found by human beings, animals and agricultural vehicles or by infected plants. It has also been observed that cysts, eggs and larvae present in the soil are carried to another place by wind (Kepenekçi, 2012). Surface water sources such as ponds, lakes, rivers and groundwater such as borehole water can harbor microorganisms that cause disease in plants. Plant pathogens can enter the water at various points in the irrigation regime, especially if the water comes into contact with plant residues or soil. Plants irrigated with water containing plant pathogens can produce disease symptoms in plants, resulting in plant death in the early seedling to sapling stage. In addition, increased use of pesticides to control diseases means increased production costs (Hong and Moorman, 2005). The first report on the presence of free-living nematodes in drinking water was reported by Tombes et al. (1979). Godfrey (1923) was the first to emphasize the possibility that plant parasitic nematodes, which cause significant yield losses in agriculture, could be distributed through irrigation water. Later, Faulkner and Bolander (1970a,b) found that 10% to 20% of the total nematode population in a main irrigation canal in Washington were plant parasites, demonstrating the potential for the spread of these parasites through irrigation water. All economically important genera of plant parasitic nematodes have been reported during surveys sampling irrigation canals, rivers, dams, runoff from agricultural fields, municipal drinking water, as well as drainage water from hydroponic systems worldwide (Cadet et al., 2002; Hong and Moorman, 2005; Hugo and Malan, 2010). Several factors such as irrigation method and timing affect microorganism transmissibility. Closed irrigation systems seem to reduce the incidence of disease incidence compared to open irrigation systems (Hoitink et al., 1992). Therefore, irrigation sources and systems need to be evaluated in terms of their contribution on plant diseases in the production system.

The prevalence of root-knot nematode species were determined in studies conducted in Isparta Province (Göze, 2014; Uysal et al., 2017). However, no study was found on the factors affecting nematode density in Isparta Province. It was determined that the studies on the determination of nematodes in irrigation sources in the world are limited in number and quite old. In Türkiye, no detailed study was found. For this reason, soil and water samples were taken from greenhouse and irrigation source to determine the effect of water sources on the presence and density of nematodes in greenhouse cultivation.

MATERIALS AND METHODS

Collection of water samples

The sampling was carried out in May and October, 2023. The areas sampled in May were sampled again in October. In Deregümü village, 20 samples were taken from different points randomly from the open pools and water wells of the general spring belonging to DSI and seen as an irrigation enterprise. GPS coordinates, time, water source and plant variety of each sample were recorded. While 13 of the samples belonged to the tomato greenhouses, 7 samples were taken from the clove greenhouses. Irrigation water samples were taken from 8 wells and 12 open pools (Table 1).

Water samples were taken using plastic bottles. One liter of water was used in each sampling. In pool sampling, a stirrer such as a stick was used to homogenize the water. Since the pool widths were not constant and the depths were not known, a fixed depth was not determined for sampling. The samples were stored in an ice box at 4ºC without being exposed to direct sunlight, extreme heat or cold and brought to the laboratory on the same day.

Table 1. Information about the sample

Code	Coordinate	Plant	Water Source
S1	N: 37°46'39.82" E:30°30'44.40"	Tomato	Well
S2	N: 37°47'03.76" E:30°30'49.48"	Tomato	Pool
S3	N: 37°47'6.19" E:30°30'9.78"	Tomato	Well
S4	N: 37°47'12.31" E:30°30'21.10"	Tomato	Pool
S5	N: 37°47'21.56" E:30°31'15.94"	Clove	Pool
S6	N: 37°47'23.23" E:30°30'10.51"	Tomato	Pool
S7	N: 37°47'30.01" E:30°30'33.39"	Tomato	Pool
S8	N: 37°47'30.34" E:30°30'33.45"	Clove	Well
S9	N: 37°47'35.7" E:30°30'58.94"	Tomato	Well
S10	N: 37°47'36.8" E:30°30'37.97"	Clove	Well
S11	N: 37°47'41.8" E:30°30'43.4"	Tomato	Well
S12	N: 37°47'44.4" E:30°30'46.58"	Tomato	Pool
S13	N: 37°47'45.336" E:30°30'41.763"	Clove	Pool
S14	N: 37°47'45.337" E:30°30'41.765"	Clove	Pool
S15	N: 37°47'52.04" E:30°31'03.20"	Tomato	Well
S16	N: 37°47'57.02" E:30°30'21.12"	Clove	Pool
S17	N: 37°47'57.65" E:30°31'13.764"	Tomato	Pool
S18	N: 37°48'13.77" E:30°31'40.67"	Tomato	Well
S19	N: 37°48'15.73" E:30°30'49.41"	Clove	Pool
S20	N: 37°48'22.97" E:30°31'42.94"	Tomato	Pool

Soil sampling

Soil samples were taken from the same place twice in May and October together with water samples in 2023 (Table 1). The samples were taken from 0-30 cm depth with a shovel in the greenhouse. Approximately 1 kg soil sample was taken from each greenhouse and placed in polyethylene bags and labeled. The samples were stored in an ice box at 4ºC without exposure to direct sunlight, extreme heat or cold and brought to the laboratory on the same day. Soil samples were kept cold in the climate chamber until analyzed in the laboratory.

Extraction of plant parasitic nematodes from water samples

The Baermann Funnel method was used to obtain nematodes from the water samples in the bottles (Hooper, 1986). The one liter bottles of each sampling were transferred to 1000 mL beakers. It was left for 24 hours for the nematodes to settle to the bottom of the water. After 24 hours, the water in the beaker was diluted to 100 ml without mixing. The remaining water in the beaker was transferred to 100 ml glass measuring cups and kept for 24 hours again for the nematodes to settle to the bottom of the water. Then it was transferred into 10 ml glass tubes and the nematodes were allowed to settle to the bottom of the water (6 hours). Then the water in the glass tube was taken from the top and the nematodes were suspended in 1 mL of water. The 1 mL of water in the glass tube was thoroughly mixed and 100 µl of water was taken from it with a micro pipette and placed on the slide, then a coverslip was placed on it and nematode genera according to Eisenback, 2002 were counted under a light microscope. After repeating this process two times, the number of nematodes found was divided into 1 mL of water and the number of nematodes found in 1 L of water was determined.

Extraction of plant parasitic nematodes form soil samples

Modified Baermann Funnel method was used to obtain plant parasitic nematodes (Hooper, 1986). The nematode genera identified according to Eisenback, 2002 and were counted under a light microscope. After

repeating this process twice, the number of nematodes found was determined by proportioning the number of nematodes found in 1 ml of water and the number of nematodes found in 100 g of soil was determined.

RESULTS AND DISCUSSION

In the soil and water samples taken in the study, 8 plant parasitic nematode genera were identified. The identified genera were: *Meloidogyne* spp., *Criconemoides* spp., *Helicotylenchus* spp., *Ditylenchus* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Xiphinema* spp. and *Tylenchus* spp. Table 2 shows the nematode genera found in the soil samples and their densities determined in May and October sampling. *Meloidogyne* spp. (S1, S6, S7, S13, S17, S20) were found in seven soil samples (Table 2). Five of these samples (S6, S7, S12, S17, S20) belonged to tomato greenhouses irrigated with pool water. Sample S13 belonged to clove greenhouse soil irrigated with pool water, while S1 was taken from tomato greenhouse irrigated with well water (Table 1). It was determined that *Meloidogyne* spp. density was higher in October (Table 2). *Criconemoides* spp. was found only in S2 in the sampling taken in May, while it was found in the soils of S2 (40 individuls/100 g soil), S14 (20 individuls/100 g soil) and S17 (20 individuls/100 g soil) samples in October. *Helicotylenchus* spp. was observed in 6 samples (S2, S7, S8, S13, S18, S19) in May, while it increased to 7 samples in October by adding S1 to these samples. The densities varied between 20-200 indivuduals/100 g soil in May, 20-680 indivuduals/100 g soil in October. Additionally, the highest density was found in S18 in May and October. While *Ditylenchus* spp. was observed in 5 samples (S3, S5, S9, S13, S19) in May, it increased to 6 samples by adding S17 to these samples in October. It is seen that the density increased in S3, S5 and S19 in October compared to May. *Pratylenchus* spp. were found in S1, S2, S8, S11, S12, S13, S16, S18, S19 samples in May and October, but their density was higher in October ranged from 80-1600 indivuduals/100 g soil. In sample S16, *Pratylenchus* spp. density in October was 4 times higher than in May. While *Paratylenchus* spp. were found in S2, S4, S13 and S19 in May sampling, they were found in 5 samples in October with the addition of S3 sample. *Xiphinema* spp. was found in soil samples S5, S13 and S14 in both May and October. In addition, *Xiphinema* spp. was detected in sample S9 in May, but not in October at the same sampling site. In sample S20, *Xiphinema* spp. was detected only in October. The highest density was determined at S14 in May (60 individuals/100 g soil) and October (80 individuals/100 g soil).*Tylenchus* spp. were found in S3, S4, S10, S13, S15, S16 and S18 in both May and October. While *Tylenchus* spp. was detected in S2 in May, it was not detected in the same sampling area in October. In sample S19, *Tylenchus* spp. was found only in October. The highest density was determined at S10 in May (600 individuals/100 g soil) and October (840 individuals/100 g soil) (Table 2).

In water samples, *Meloidogyne* spp. were detected in S1, S6, S7, S17 and S20 in May, while S9, S12, S13 and S18 were added to these samples in October. It is also observed that the density was high in October. While *Criconemaides* spp. was detected only in water sample S2 in May, it was detected in water samples S2, S14 and S17 in October. The highest density was found in S2 in May (100 individuals/1 l water) and October (200 individuals/1 l water). *Helicotylenchus* spp. was found in 6 of the water samples (S2, S7, S8, S13, S18, S19) in May, while it increased to 7 with S1 in October. In the S8 sample, it was found that the density increased to 800 individuals/1 l water in October, the highest among those detected. *Ditylenchus* spp. was found in S1, S2, S5, S13 and S19 water samples in May, while it was also found in S3 and S11 samples in October. *Ditylenchus* spp. density was higher in S2 and S5 water samples than the others. In sample S5, density in October was 4 times higher than in May. In May, *Pratylenchus* spp. was found in water samples S16, S18 and S19, while in October it was also found in water samples S3 and S15. The density of *Pratylenchus* spp. was higher in water sample S19 than in the other samples. In water sample S19, *Paratylenchus* spp. was not detected in May, while it was detected in October. The density of *Paratylenchus* spp. found in S2 water sample in October was 7 times higher than in May. *Xiphinema* spp. was found only in water samples S14 and S19 in May, while it was found in water samples S2, S3, S13, S14 and S17 in October. The highest density was determined at S14 in May (100 individuals/1 l water) and October (180 individuals/1 l water). *Tylenchus* spp. was detected in S4, S10, S13, S15 and S18 water samples in May, while it was detected in S1, S4, S10, S13, S15 and S18 water samples in October. *Tylenchus* spp. density in water sample S13 was higher than the others (Table 3).

Criconemoides spp. were detected in both soil and water samples of samples S2, S14 and S17. In sample S5, *Criconemoides* spp. was found only in water. *Helicotylenchus* spp. were detected in both soil and water of samples S1, S2, S8, S13, S18 and S19. *Helicotylenchus* spp. were found in water samples of S3, S11 and S15, but not in soil samples. On the other hand, *Helicotylenchus* spp. was found in the soil of sample S7, but not in the sample taken from the irrigation source. *Ditylenchus* spp. was found only in the water sample in S1 and S2, but only in the soil sample in S9 and S17. *Ditylenchus* spp. was found in soil and water samples of S3, S5, S13 and S19. *Pratylenchus* spp. was found only in soil samples of S1, S2, S8, S11, S12 and S13, but only in water samples of S3 and S15. *Pratylenchus* spp. was found in both soil and water samples of S16, S18 and S19. *Paratylenchus* spp. was found only in the soil sample of S3, while it was detected only in the water sample of S6. *Paratylenchus* spp. were detected in both soil and water samples of S2, S4, S13 and S19. *Xiphinema* spp. was found only in water samples of S2, S3, S17 and S19, while it was found only in soil samples of S5, S9 and S20. *Xiphinema* spp. was found in soil and water samples of S13 and S14. While *Tylenchus* spp. was found only in water in S1, it was found

only in soil in S2, S3, S16 and S19. *Tylenchus* spp. were detected in soil and water samples in S4, S10, S13, S15 and S18 (Table 4).

Table 2. Plant parasitic nematodes detected in soil samples and their densities in Deregümü tomato and clove greenhouses

Code	Density of plant parasitic nematode genera in 100 g soil															
	***Meloidogyne* spp.**		***Criconemoides* spp.**		***Helicotylenchus* spp.**		***Ditylenchus* spp.**		***Pratylenchus* spp.**		***Paratylenchus* spp.**		***Xiphinema* spp.**		***Tylenchus* spp.**	
	M	**O***	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**
S1	50	100	-	-	-	20	-	-	100	80	-	-	-	-	-	-
S2	-	-	20	40	60	80	-	-	60	200	20	20	-	-	20	-
S3	-	-	-	-	-	-	20	60	-	-	-	40	-	-	40	60
S4	-	-	-	-	-	-	-	-	-	-	200	460	-	-	60	100
S5	-	-	-	-	-	-	100	140	-	-	-	-	20	20	-	-
S6	120	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S7	60	800	-	-	120	240	-	-	-	-	-	-	-	-	-	-
S8	-	-	-	-	100	480	-	-	300	520	-	-	-	-	-	-
S9	-	-	-	-	-	-	240	180	-	-	-	-	20	-	-	-
S10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	600	840
S11	-	-	-	-	-	-	-	-	380	460	-	-	-	-	-	-
S12	20	100	-	-	-	-	-	-	120	400	-	-	-	-	-	-
S13	100	900	-	-	20	60	40	40	80	360	20	80	20	20	100	140
S14	-	-	-	20	-	-	-	-	-	-	-	-	60	80	-	-
S15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	40
S16	-	-	-	-	-	-	-	-	400	1600	-	-	-	-	20	20
S17	40	480	-	20	-	-	-	20	-	-	-	-	-	-	-	-
S18	-	-	-	-	200	680	-	-	120	560	-	-	-	-	60	240
S19	-	-	-	-	20	60	60	400	140	680	40	60	-	-	-	20
S20	480	720	-	-	-	-	-	-	-	-	-	-	-	20	-	-

*M: May, O: October

Table 3. Plant parasitic nematodes and their densities detected in irrigation sources of Deregümü tomato and clove greenhouses.

Code	Density of plant parasitic nematode genera in 1 L water															
	***Meloidogyne* spp.**		***Criconemoides* spp.**		***Helicotylenchus* spp.**		***Ditylenchus* spp.**		***Pratylenchus* spp.**		***Paratylenchus* spp.**		***Xiphinema* spp.**		***Tylenchus* spp.**	
	M	**O***	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**	**M**	**O**
S1	140	200	-	-	-	200	140	380	-	-	-	-	-	-	-	140
S2	-	-	100	200	100	100	800	1200	-	-	400	2800	-	100	-	-
S3	-	-	-	-	260	-	-	180	-	20	-	-	-	20	-	-
S4	-	-	-	-	-	-	-	-	-	-	200	460	-	-	60	100
S5	-	-	20	20	-	-	1000	4000	-	-	-	-	-	-	-	-
S6	100	300	-	-	-	-	-	-	-	-	40	100	-	-	-	-
S7	840	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S8	-	-	-	-	620	800	-	-	-	-	-	-	-	-	-	-
S9	-	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	500
S11	-	-	-	-	140	220	-	20	-	-	-	-	-	-	-	-
S12	-	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S13	-	1400	-	-	600	480	20	100	-	-	120	160	-	100	1000	2600
S14	-	-	-	20	-	-	-	-	-	-	-	-	100	180	-	-
S15	-	-	-	-	400	-	-	-	-	20	-	-	-	-	20	20
S16	-	-	-	-	-	-	-	-	800	980	-	-	-	-	-	-
S17	180	320	20	20	-	-	-	-	-	-	-	-	-	100	-	-
S18	-	500	-	-	200	680	-	-	120	560	-	-	-	-	60	240
S19	-	-	-	60	20	20	100	400	720	1240	-	60	20	-	-	-
S20	480	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*M: May, O: October

Table 4. Plant parasitic nematodes detected in soil and water samples

Code	*Criconemoides* spp.				*Helicotylenchus* spp.				*Ditylenchus* spp.				*Pratylenchus* spp.				*Paratylenchus* spp.				*Xiphinema* spp.				*Tylenchus spp.*			
	Soil		Water		Soil		Water		Soil		Water		Soil		Water		Soil		Water		Soil		Water		Soil		Water	
	M*	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O
S1	-	-	-	-	-	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
S2	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	-	-	-
S3	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	+	-	+	-	-	-	-	-	+	+	+	-	-
S4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
S5	-	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
S6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
S7	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S8	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S9	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
S10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
S11	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S12	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S13	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+
S14	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
S15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+
S16	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-
S17	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
S18	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+
S19	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	-
S20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

* M: May, O: October, +: Found, -: Not found

Table 5. Comparison of root-knot nematode densities in soil and water samples

Code	Plant	Water Source	Soil (100 g)		Water (1 l)	
			May	October	May	October
S1	Tomato	Well	50	100	140	200
S2	Tomato	Pool	-	-	-	-
S3	Tomato	Well	-	-	-	-
S4	Tomato	Pool	-	-	-	-
S5	Clove	Pool	-	-	-	-
S6	Tomato	Pool	120	400	100	300
S7	Tomato	Pool	60	800	840	400
S8	Clove	Well	-	-	-	-
S9	Tomato	Well	-	-	-	500
S10	Clove	Well	-	-	-	-
S11	Tomato	Well	-	-	-	-
S12	Tomato	Pool	20	100	-	200
S13	Clove	Pool	100	900	-	1400
S14	Clove	Pool	-	-	-	-
S15	Tomato	Well	-	-	-	-
S16	Clove	Pool	-	-	-	-
S17	Tomato	Pool	40	480	180	320
S18	Tomato	Well	-	-	-	500
S19	Clove	Pool	-	-	-	-
S20	Tomato	Pool	480	720	480	500

Meloidogyne spp. was found in both soil and water samples of S1, S6, S7, S12, S13, S17 and S20, but only in water samples of S9 and S18. *Meloidogyne* spp. was detected in only one of the 7 clove samples (S13), while *Meloidogyne* spp. was detected in 7 of the 13 tomato samples. While one of the tomato greenhouses was detected in well irrigation source, 6 of them were identified as pools. It was observed that the likelihood of finding root knot nematode was higher in pool samples. In soil and water samples, *Meloidogyne* spp. density was higher in October. The soil densities varied between 100-900 individuals /100 g soil in October, they varied between 200-1400 individuals/1 l water in water samples. Only S7 sample, the water density of May (840 individuals/1 l water) was higher than October (400 individuals/1 l water). Additionally, the highest density was found in S13 (clove) with 900/100 g soil and 1400/l water (Table 5).

Plant parasitic nematode groups causing economically important damages were detected in soil and irrigation water samples taken in this study. The most important group among these was the root knot nematodes (*Meloidogyne* spp.). The prevalance of root knot nematodes was higher in tomato samples than cloves. In previous studies conducted in Isparta Province, it was reported that root knot nematodes were common in greenhouses (Kepenekçi et al., 2012; Göze 2014, Uysal et al., 2017). Thomason and Van Gundy (1961) detected two *Meloidogyne* species in the roots of weeds growing on the banks of the Colorado River and in direct contact with water. In addition, clove growers are large companies, and during the interviews, it was determined that they applied fumigation under cover for a short time (15-20 days). Root knot nematode were also detected in the water of all samples in which root knot nematode was found in the soil. In S9 and S18, where root knot nematode was

detected only in water samples, not found in soil samples. The fact that these nematodes were not found in either soil or water samples in May, but were found in the water sample taken in October suggests that the transport factor emerged due to the increasing nematode density. Root-knot nematodes were found more in pool irrigation water than in well water. This may be due to plant-soil contact since the pools are open. Heald and Johnson (1969) reported that pressure in the nozzles or pump could injure the larvae. While agricultural land irrigated with canal irrigation water was found to be heavily infested with nematodes, no plant parasitic nematodes were found in irrigated with water from wells (Faulkner and Bolander 1970b). This research is in agreement with Hong and Moorman (2005) who reported that water from wells can generally be considered free of plant parasitic nematodes. However, they noted that if the well is not sealed, flowing water carrying sediment contaminated with plant parasitic nematodes can enter.

Other plant parasitic nematode genera identified in the study were *Criconemoides* spp., *Helicotylenchus* spp., *Ditylenchus* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Xiphinema* spp. and T*ylenchus* spp. The population levels of plant parasitic nematodes in soil and water samples were correlated. Such a finding suggests that different species react differently in terms of their presence or distribution through flowing water. *Hoplolaimus* spp., *Tylenchorhynchus* spp. and *Criconemoides* spp. were only detected in the bottom sediments of ponds (Smith and van Mieghem, 1983). In South Africa, the spread of *Xiphinema index* along the Breede River from the Robertson and Bonnievale areas is most likely the result of irrigation directly from this river (Barbercheck et al., 1985). *Criconemoides, Helicotylenchus, Pratylenchus, Meloidogyne, Tylenchorhynchus, Hoplolaimus* and *Trichodorus* genus were detected in pond water in Georgia, USA (Heald and Johnson, 1969). *Gracilacus parvula, Helicotylenchus dihystera, Pratylenchus pseudopratensis, Scutellonema caveness, Tylenchorhynchus gladiolus* and *T. mashoodi* were identified in samples taken from running water (Cadet et al., 2002). *Criconemoides* spp. have a wide range of hosts including field crops, fruit trees, ornamentals, vegetables, nurseries, shrubs, grasses, perennial woody plants and weeds. However, the host status of parasitized plants is not fully known (Siddiqi, 2000).The main route of long and short distance spread of Criconemoides species is through artificial movement of infected species. It can also spread into regions through contaminated production material, contaminated soil, agricultural implements and machinery, water runoff, irrigation and human activities (Haque and Khan, 2021). *Xiphinema* spp. is economically damaging plant parasitic nematode genera to grapes, hops and strawberries. Other documented hosts include: nectarine, oak, rose, vine, raspberry, carrot, cherry, peach and soybean (Nemaplex, 2024). *Xiphinema* species have been reported to transmit the virus (Jones et al., 2013). The presence of *Xiphinema* spp. in tomatoes and cloves under cover indicates waterborne transmission. Fruit cultivation and vineyards are also common in Deregümü district. Contaminated plant residues and soil may have been mixed into the pond water. *Xiphinema* spp. reported mostly in irrigation water coming from irrigation canals (Faulkner and Bolander 1970a; Waliullah, 1984,1989; Roccuzzo and Ciancio, 1991). It has also been found to be found in flowing water, rivers and dams (Heald and Johnson 1969; Smith and van Mieghem, 1983).

As a result of this study, the effect of water sources on nematode carriage and nematode prevalence and density in the greenhouse were determined. It has been determined that irrigation with an open pool is risky. Contact of pools with contaminated plant and soil residues should be prevented.

CONCLUSION

More work is needed on plant pathogens, including nematodes in irrigation water. A wide variety of organisms can be found in water. Scientists will then need to carry out research to control them. Management strategies should be designed to suit each water source. Since water does not naturally contain nematodes, preventive measures must be taken to keep it nematode-free and limit its ability to act as a source of transport for plant parasitic nematodes, which then act as a contaminant of valuable and limited agricultural soils.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

Concept: FGGÖ; Data Collection and/or Processing: EE, HÇ; Analysis and/or Interpretation: FGGÖ, EE, HÇ; Literature Search: FGGÖ, EE; Writing Manuscript: FGGÖ, HÇ; Critical Review: FGGÖ. The authors 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.

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