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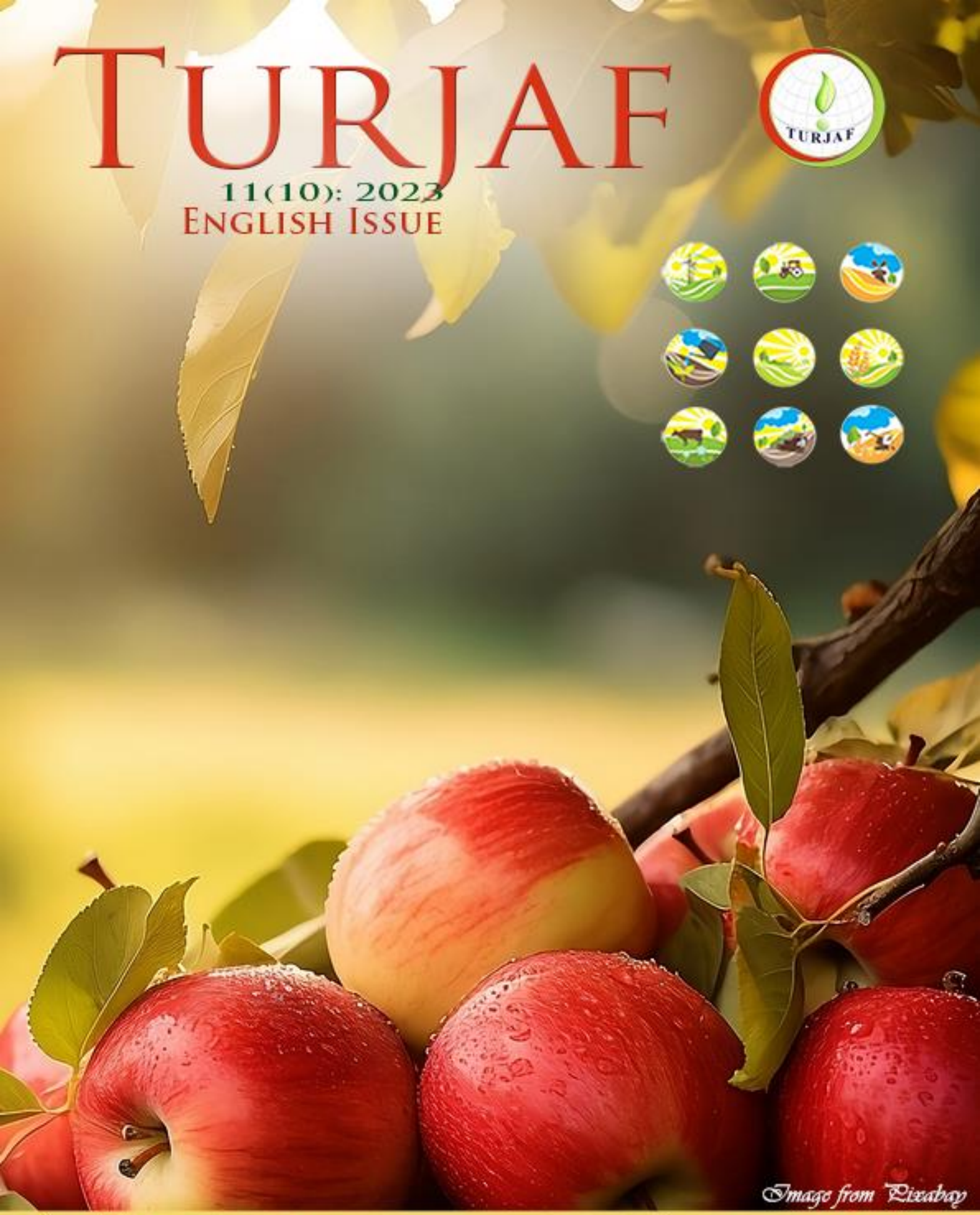

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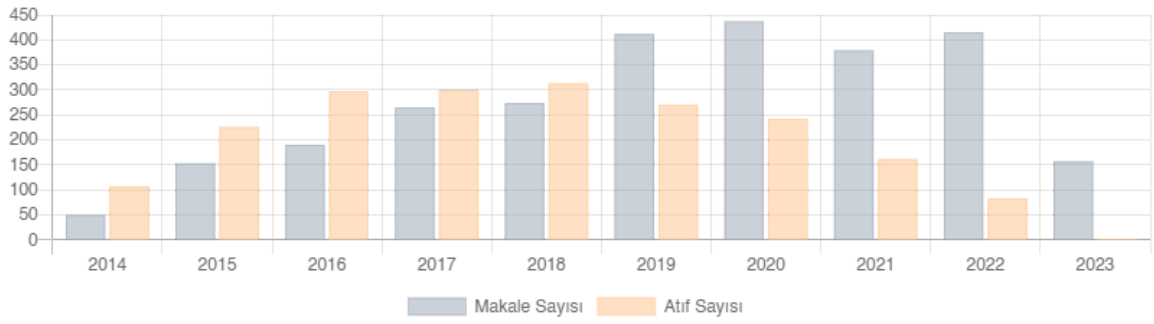

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Screening of Natural Deep Eutectic Solvents for the Recovery of Valuable Phenolics From Waste of Shalgam Juice Process

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ABSTRACT

Shalgam juice is one of the most popular non-alcoholic beverages in Türkiye and also in many countries. The high amount of production, regarding the high amount of consumption leads to an inevitable amount of solid waste. This amount reaches to almost 12 million kg of fermented black carrot annually. The accumulation of this waste causes fatal environmental pollution. The waste of shalgam juice process, fermented black carrot, retains significant amount of valuable components, such as phenolics, flavonoids, and anthocyanins. In this study new generation green solvents, Natural Deep Eutectic Solvents (NADESs) were screened for the extraction of valuable phenolics from fermented black carrot using ultrasound assisted extraction. The NADESs used were selected from four different groups such as acidic, sugar-based, choline chloride-sugar based and polyol-based. According to the results, the members of polyol and choline chloride sugar NADESs showed up. Choline chloride-glycerol (polyol group), extracted the highest total phenol amount (16.04 mg/g) and also provided the highest antioxidant activity (81.77%). On the other hand, NADESs belonging to choline chloride-sugar group were effective for the extraction of flavonoids and monomeric anthocyanins. Namely, choline chloride-fructose-water extract contained 21.45 mg/g of total flavonoids, while choline chloride-sucrose-water extract contained 1680.51 mg/kg of total monomeric anthocyanins. The performances of NADESs tested were found to be higher than that of water and ethanol showing the high yield recovery of valuable phenolics with NADESs. The results exhibited the significance of the components inside the waste. The remaining valuable content could be easily and efficiently extracted using NADESs and these extracts –as a mixture or after purification- can furtherly be used for different purposes in different fields, such as cosmetics, antioxidant preparations, etc, using a zero-cost waste as the input of the processes.

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Introduction

Shalgam juice is one of the most popular non-alcoholic beverages in Türkiye. The bitter-sour taste matches perfectly with the traditional local food therefore, it is preferred together with almost every meal (Canbaş and Fenercioğlu, 1984). It is a savory beverage and helps with the digestion system. Since ancient times people have prepared this beverage by their own fermentation methods, especially in Adana, Mersin and Hatay –in the south part of Türkiye (Canbaş and Fenercioğlu, 1984). Nowadays the consumption of shalgam juice is widespread in Türkiye and it is commercially produced at high amounts and exported to more than 30 countries (*Dünya şalgam suyunu keşfetti*, 2020). The production of shalgam juice in Türkiye was reported as 60 million liters per year in 2020 and increased to 70 million liters per year in 2022 which is quite high (*Dünya şalgam suyunu keşfetti*, 2020; Ekinci, 2022). This high amount of production leads to an inevitable amount of solid waste that corresponds to almost 20% of the juice produced (Tanrıseven et al., 2018) that is about 12 million kg of fermented black carrot annually. The accumulation of this waste causes environmental pollution in terms of stench and the physical presence of the waste.

Due to the increasing consciousness about the environmental pollution researchers struggle for the evaluation of the wastes which cannot be prevented. In this aspect, some types of the waste, especially in the food industry have been investigated in order to be reevaluated for their potential to be a zero-cost waste as an input of many different processes in other fields (Ahmad and Danish, 2018; Cheok et al., 2018).

Fresh black carrot is a well-known source of various types of phenolics such as anthocyanins, carotenoids, flavonoids, antioxidants, vitamins, and etc. (Khandare et al., 2011; Akhtar et al., 2017). In the production process of shalgam juice, black carrots are fermented in the tanks in the form of chopped pieces in 2-3 cm in length. The thick form of the pieces lets the vegetable to retain various beneficial components inside during fermentation process.

In the literature there are many studies that reported the evaluation of bioactive compounds from fresh black carrot (Türker and Erdoğdu, 2006; Ersus and Yurdagel, 2007; Gizir et al., 2008; Gras et al., 2015; Guldiken et al., 2016) but there are only a few studies on the extraction of the waste of black carrot from processing plants (Ağçam and Akyıldız, 2015; Agcam et al., 2021). Most of these studies use conventional extraction processes that require the utilization of abundant organic solvents. One of these studies used acidified methanol, ethanol and water for the extraction of anthocyanins from black carrot waste and reported high amount of total monomeric anthocyanins (Ağçam and Akyıldız, 2015). They also reported that the acidity of these solvents was effective on the recovery. Despite the high yields provided by organic solvents, the harms of these processes to the environment are inevitable. On the other hand, organic solvent free extraction was also performed, however, the system used required high pressure, that resulted in high cost (Agcam et al., 2021).

Increasing consciousness on the environmental and health issues directed researchers to the utilization of green processes. One of the most popular green strategies involves the replacement of organic solvents with green solvents. A significant class of green solvents are Deep Eutectic Solvents (DESs). They are regarded as new generation green solvents and they have been investigated to replace organic solvents in many fields (Tang et al., 2015; Li and Row, 2016; Cunha and Fernandes, 2018; Ünlü, 2021; Deniz et al., 2023). Besides their advantages such as easy preparation, low volatility, non-toxicity, high solvation capacity; the recent research conducted on the synthesis of different DESs resulted in a new sub-class that are called Natural Deep Eutectic Solvents (NADESs) (Dai et al., 2013b; Paiva et al., 2014; Vanda et al., 2018). The additional advantage of these new solvents were declared as the natural structure of the constituents. Their natural substance origin makes them attractive for researchers especially in the various extraction processes (Tang et al., 2015; Cvjetko Bubalo et al., 2016; Li and Row, 2016; Peng et al., 2016; Ruesgas-Ramón et al., 2017; Cunha and Fernandes, 2018; Meng et al., 2018; Ünlü, 2021; Deniz et al., 2023).

Among the non-conventional procedures, ultrasound assisted extraction takes attention as a simple, cheap and effective process (Huang et al., 2009). During exposure to ultrasound waves, cavitation is increased and effective mass transfer is achieved (Knorr et al., 2002). Additionally, ultrasound-assisted extraction was reported to be safe for heat sensitive components (Tiwari, 2015).

In this study, we aimed to reveal the potential of fermented black carrot that is the waste of shalgam juice process in terms of valuable ingredients that remains inside even after fermentation. In accordance with the aim to evaluate the waste for a sustainable process, toxic organic solvent utilization was avoided in this study. Instead, eleven different NADESs were used as green, natural and effective extraction solvents. Ultrasound waves were utilized in order to achieve high yield extractions at mild conditions. We evaluated the presence of phenolics in terms of total phenol amount, total flavonoid amount, total monomeric anthocyanin amount and presented antioxidant activities of the extracts. To our knowledge this is the first study to evaluate the waste of shalgam juice plant using NADESs as green solvents.

Materials and Methods

Preparation of Shalgam Juice Waste

Fermented black carrot was obtained from Yeni Kavaklıdere Company (Ankara, Turkey) with no cost. Solid waste was ground by blender (Braun MQ9078X) and lyophilized at 0.04 mbar and -50 °C (Hetosicc,CD 52-1, Heto Lab). The particles were then separated using molecular sieves (Endecotts, Octagon 200, England) and 425 µm-1 mm sized particles were used for the extraction processes.

Preparation of NADESs

Required amount of the components (Table 1) were weighed in a screw-capped bottle and heated (50, 60 or 80°C) for 2h, till clear liquid was formed. Choline chloride was dried under vacuum over silica gel in a desiccator prior to use. The NADESs that were prepared, their abbreviations and synthesis temperatures were given in Table 1.

Extraction of Shalgam Juice Process Waste

For the extractions, a modified method for anthocyanin extraction was used (Bubalo et al). 12 ml of total volume was added on 0.8 g of grounded lyophilized waste. NADESs were used as a mixture with water at 50% (v/v), to increase the polarity and also decrease the viscosity in order to provide easy handling (Altundağ et al., 2021; Ünlü, 2021; Deniz et al., 2023). Experiments was conducted using an ultrasonic water bath with a plug-in temperature controller, at 50 °C for 30 min (Elma S30H, Singer, Germany) with a frequency of 37 kHz and a power of 140W. After extraction, the extract was filtered through a 0.45 µm syringe filter and stored at -30 °C in the dark.

Total Phenol Amount

Total phenolic amounts of the samples were determined by the Folin-Ciocalteu method. 20 µL of extracts were mixed with 1580 µL of distilled water and 100 µL Folin-Ciocalteu reagent was added. After 1 min of incubation, 300 µL of 20% Na_2CO_3 was added and vortexed. The mixture was incubated at room temperature in the dark for 2h. At the end of the incubation period the absorbance was read at 750 nm. The results are given as gallic acid equivalent (Arnous et al., 2001).

Total Flavonoid Amount

Total flavonoid amounts of extracts were measured as reported by Choi et al. (2011). 100 µL of extract, 1000 µL of diethylene glycol and 100 µL of NaOH were mixed in a test tube and incubated at 37 °C for 60 min. The absorbance was measured at 420 nm. The concentrations of flavonoids were presented in quercetin equivalent (Choi and Kim, 2011).

Total Monomeric Anthocyanin Amount

Total monomeric anthocyanin amount was determined using pH differential method (Giusti and Wrolstad, 2001). Samples were diluted with 0.025 M potassium chloride buffer (pH=1) and with 0.4 M sodium acetate buffer (pH=4.5). After equilibration for 15 min, absorbances at λ_{max} (527 nm) and 700 nm were measured. Total monomeric anthocyanin concentration (TMAP) was calculated as cyanidin-3-glucoside equivalents by

following equation in mg/L; then corresponding amount (mg/kg) was calculated with the use of solid-liquid ratio.

$$\text{TMAP}\left(\frac{\text{mg}}{\text{liter}}\right) = \frac{A \times MW \times DF}{\varepsilon \times 1} \times 100$$

Where;

$A = (A_{527nm} - A_{700nm})_{pH1} - (A_{527nm} - A_{700nm})_{pH4.5}$

MW (molecular weight)= 449.2 g/mol for cyanidin-3-glucoside

DF= Dilution factor

l= path length in cm

ε= 26900 molar extinction coefficient in $L x mol^{-1} \times cm^{-1}$ for cyanidin-3-glucoside

1000= Factor for conversion from g to mg.

Antioxidant activity

Antioxidant activities were measured using DPPH radical scavenging method (Fernando and Soysa, 2015). 60 µL extract was mixed with 1140 µL of 100 µM DPPH (in ethanol). The mixture was kept at room temperature in the dark for 30 min. The absorbance at 517 nm was recorded (A_{sample}). As control, the same procedure was carried out without sample ($A_{control}$). The percentage of DPPH scavenging effect (inhibition %) was calculated by following equation.

$$\text{Inhibition}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical analysis

All extractions and analysis were performed in triplicates and average values were presented. The results were analyzed by one-way analysis of variance (ANOVA) and t-test and $P<0.05$ were considered significantly different using GraphPad Prism Version 10.0.2 (GraphPad Software, Inc, La Jolla, CA, USA).

Results

NADESs used in this study were selected based on our previous studies such as extraction of phenolics from olive leaves (Ünlü, 2021) or apple pomace (Deniz et al., 2023). The broad range of NADESs let us the to screen the relevant types on a general basis. Regarding this, eleven green solvents from four different groups, as acidic, sugar based, choline chloride-sugar based and polyalcohol based NADESs were tested using one-pot-at-a-time method (Table 1).

Total Phenolic Amount

Figure 1 shows total phenolic amounts obtained using eleven different NADESs and also reference extractions performed using water and EtOH. According to the results the highest phenolic amount was achieved using CG (1:2) as 16.04 ± 0.87 mg/g, followed by CFW (5:2:5) as 14.23 ± 0.42 mg/g. On the other hand, total phenolic amounts obtained for GFW (1:1:11), CLa (1:9), GSW (1:1:11), CEG (1:2) were found to be around 10 mg/g (differences were not significant; $p>0.05$). Regarding different groups of NADESs, CG (1:2) was found to be the best in polyol group, CLa (1:9) in acidic group, GFW in sugar group and CFW (5:2:5) in choline-chloride sugar group. On the other hand, it was found that NADESs could extract higher amount of phenolics than water (6.29 ± 0.34 mg/g) and EtOH (2.50 ± 0.66 mg/g).

Total Flavonoid Amount

The changes in total flavonoid amounts with different NADESs were presented in Figure 2. The highest flavonoid amount was obtained as 21. 45 ± 0.10 mg/g using CFW (5:2:5) which was almost 20% higher than the closest value obtained with GFW (1:1:11) (17.38 ± 0.70 mg/g). NADESs that provided the highest flavonoid amounts from each group were; CFW (5:2:5), GFW (1:1:11), CLa (1:9), CEG (1:2), from choline chloride sugar, sugar, acidic and polyol groups, respectively. Interestingly, three members from the sugar group of NADESs-except for GFSW (1:1:1:11) - lined up as second, third and fourth to extract the flavonoids. Among the acidic group, molar ratio of 1:9 showed up, however, no correlation could be detected between the extracted flavonoid amount and lactic acid molar ratio in NADES. On the other hand, water and EtOH were not effective on the extraction of flavonoids.

Total Monomeric Anthocyanin Amount

When total monomeric anthocyanin amounts are evaluated, CSW (4:1:4) stood out as shown in Figure 3, providing the highest monomeric anthocyanin amount as 1680.51 ± 22.21 mg/kg. Monomeric anthocyanins could be extracted at higher amounts using polyol and sugar containing NADESs whereas acidic NADESs were not effective in this aspect. However, total monomeric anthocyanin amount decreased with increasing amount of lactic acid in NADESs. On the other hand, water was found to extract anthocyanins at the sixth order, whereas EtOH was at the last order.

Table 1. List of NADESs prepared

NADES group	Name	Abbreviation	T	Reference
Acidic	Choline chloride-lactic acid	CLa (1:1)	60 °C	(Jablonský et al., 2018)
		CLa (1:6)	60 °C	(Jablonský et al., 2018)
		CLa (1:9)	60 °C	(Jablonský et al., 2018)
Sugar based	Glucose-fructose-sucrose-water	GFSW (1:1:1:11)	50 °C	(Mohammadpour et al., 2018)
	Glucose-fructose-water	GFW (1:1:11)	50 °C	(Mohammadpour et al., 2018)
	Glucose-sucrose-water	GSW (1:1:11)	50 °C	(Mohammadpour et al., 2018)
	Fructose-sucrose-water	FSW (1:1:11)	50 °C	(Mohammadpour et al., 2018)
Choline chloride-sugar based	Choline chloride-fructose-water	CFW (5:2:5)	80 °C	(Elgharbawy, 2018)
	Choline chloride-sucrose-water	CSW (4:1:4)	80 °C	(Elgharbawy, 2018)
Polyol-based	Choline chloride-ethylene glycol	CEG (1:2)	80 °C	(Zhang et al., 2012)
	Choline chloride- glycerol	CG (1:2)	80 °C	(Dai et al., 2013a)

T: Temperature

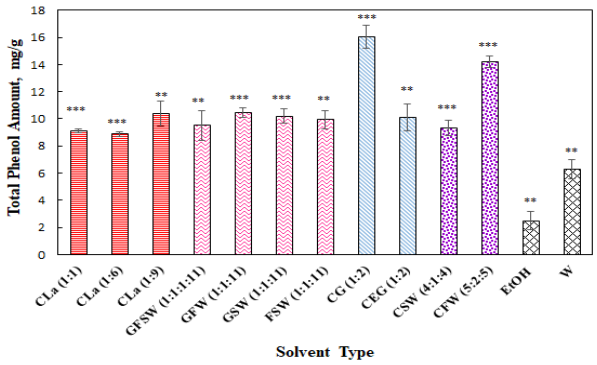

Figure 1. The effect of solvent types on total phenol amount ($p \leq 0.001$: ***, $p \leq 0.01$: **)

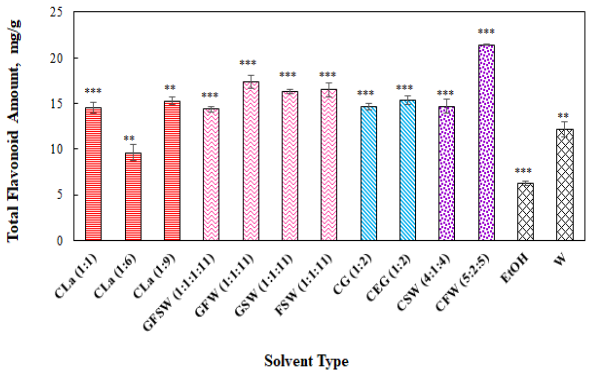

Figure 2. The effect of solvent types on total flavonoid amount ($p \leq 0.001$: ***, $p \leq 0.01$: **)

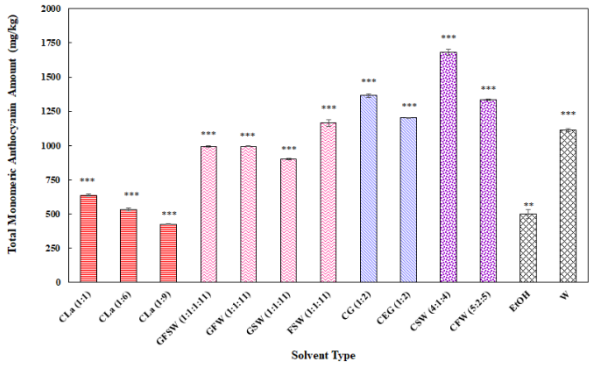

Figure 3. The effect of solvent types on total monomeric anthocyanin ($p \leq 0.001$: ***, $p \leq 0.01$: **)

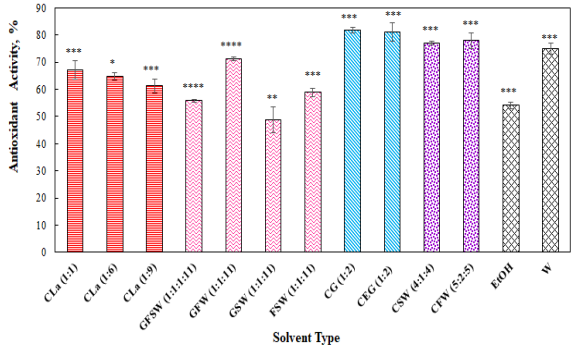

Figure 4. Antioxidant activities of the extracts ($p \leq 0.001$: ***, $p \leq 0.01$: **, $p \leq 0.05$: *)

Antioxidant Activity

Antioxidant activities of the extracts were investigated using DPPH as the radical. Control absorbance value to be used in Equation (1) was measured using NADESs without the extracts to eliminate the potential interference of the solvents with the procedure. The range of the antioxidant activities was found to change in the range of 81.77-48.63% (Figure 4). According to the results, the highest two values were very close to each other as 81.77± 1.01% and 81.22 ± 0.99% provided by CG (1:2) and CEG (1:2)), respectively. The extracts generally showed high antioxidant activities. Interestingly, antioxidant activity values decreased with increasing amount of lactic acid in NADESs, similar to monomeric anthocyanins.

Discussion

In this study, waste of shalgam juice was extracted using eleven different NADESs mostly selected from of our previous research (Ünlü, 2021; Deniz et al., 2023). Choline chloride sugar and sugar containing NADESs such as CFW (5:2:5), GFW (1:1:11) and CSW (4:1.4) were found to be prominent solvents. When the results are compared with the literature, it was reported that total phenol and flavonoid of olive leaves were extracted the most by choline-chloride sugar containing NADES; CFW (5:2.5) (Ünlü, 2021). On the other hand, GSW (1:1:11) as a sugar group NADES was declared to show up as the green solvent to extract phenolics from apple pomace (Deniz et al., 2023). Hence, similar findings of the extractions from different wastes revealed that sugar/choline chloride sugar group NADESs may be regarded as effective green solvents for phenolic components.

On the other hand, polyol group NADES; CG (1:2) extracted the highest amount of total phenols from the waste; fermented black carrot and also provided the highest antioxidant activity. When compared to the other member of polyol group that is CG (1:2); CEG (1:2) has extra hydroxyl group in the structure which might have positive effect on the efficiency of the extraction.

To observe the effect of the lactic acid content of NADESs, three different molar ratios of choline chloride-lactic acid NADESs were prepared. Total flavonoid amount and antioxidant activity results were inversely proportional to the increase in lactic acid amount of NADESs while total phenol and flavonoid amounts could not be correlated with the amount of lactic acid in NADES. On the contrary, the highest lactic acid containing NADES extracts contained the highest phenol and flavonoid amounts, while the lowest lactic acid containing NADES extracts were found to have the highest total monomeric anthocyanin and antioxidant activity. In contrast, apple pomace flavonoids were reported to be proportional to lactic acid content of NADES (Deniz et al., 2023).

Apart from NADESs, water and EtOH were also used to highlight their performances as commonly used solvents. Contrary to expectations, water was found to extract higher amounts of phenolics than ethanol as shown in Figs 1-3. This may be due to one of the ingredients of shalgam juice process; NaCl, that might have facilitated the extraction of phenolics from the waste. In the literature, the increase in the NaCl concentration in water was reported to increase the solubility of some phenolic compounds

(Noubigh et al., 2007). On the contrary, NaCl content of shalgam juice might have caused a negative effect on the extraction of phenolics by ethanol, since NaCl was reported to have quite low solubility in ethanol (Pinho and Macedo, 2005).

The results of the total phenolic amount could only be compared with Agcam et al. (2021) as this was the only study to present the phenolic amounts from the waste of black carrot pomace. The authors used a high pressure treatment system for the pomace-water mixture and also propylene glycol to transfer the pressure. They reported 350.93 mg/L of total phenolic amount and 107.0 mg/L of total monomeric anthocyanin at around 300 MPa and 60-80 °C (Agcam et al., 2021). Considering the discrepancy of the extraction methods one-to-one comparison would be inadequate, however, comparison of the highest total phenol and flavonoid amounts together with the cost of the systems used, one can conclude the present method as more advantageous, since almost three-fold higher total phenolic amount (CG (1:2) - 1067 mg/L, (16 mg/g)) could be obtained with a lower cost procedure. Total monomeric anthocyanins were compared to a study that presented vortex assisted acidified organic solvent extraction (Ağçam and Akyıldız, 2015). The range of total monomeric anthocyanins were reported as 656.2 - 1191.9 mg/kg. Compared to our results one can conclude that ultrasound assisted NADES extraction yielded almost 1.5-fold higher amount of total monomeric anthocyanins (1680.51 ± 22.21 mg/kg).

To highlight the amount of the valuable components retained inside the fermented black carrot as the waste of shalgam juice, examples of the extractions from fresh black carrot are presented below.

Jabbar et al. (2015) optimized the extraction of fresh black carrot using ultrasonic waves and organic solvents (Jabbar et al., 2015). They reported 316.74 µg/g phenolic amount at the optimized conditions (17 min, 34 °C, %48 EtOH) which is quite low than the results obtained in this study (16000 µg/g). On the other hand, Kumar et al. (2019) used microwave assisted extraction with EtOH and reported considerably higher phenolic amount as 264.9 ± 10.02 mg GAE/100 mL which is almost two-fold of the waste of shalgam juice (Kumar et al., 2019). Türker and Doğan (2021) used five different DESs to extract monomeric anthocyanins from fresh black carrot by ultrasound assisted extraction and the highest amount was obtained using choline chloride-citric acid (1:1) DES as around 6 mg/g (Aslan Türker and Doğan, 2021). This value is almost three-fold higher than the waste of shalgam juice. Hence we can conclude that roughly, one third to half of the phenolics in the fresh carrot remain inside the waste.

Conclusion

The waste; fermented black carrot was obtained from shalgam juice plant with no cost. After some preliminary steps to protect the waste from spoiling ultrasound-assisted NADES extraction was performed to recover valuable components. This study reveals the first time screening of NADESs in the extraction of valuable components from shalgam juice waste. With this aim, total phenol, flavonoid, monomeric anthocyanin amounts, as well as antioxidant activities were presented. Though the best solvent for each parameter varied, a general conclusion could be exhibited as, among the different groups tested, sugar / choline chloride sugar containing NADESs and also a member of polyol group NADES, that is CG(1:2) came forward. On the bright side, when compared to different extraction strategies, the green route presented in this study provided higher amounts of phenolics. The advantages of the presented procedure are; lower cost strategy than a high pressure process and a greener alternative to acidified organic solvents, besides, the procedure is effective and mild. So that the presented procedure has the potential to be an opt for the extraction of shalgam juice waste. On the other hand, flavonoid amounts and antioxidant activities were brought in as additional data to the literature. Additionally, hidden and/or neglected potential of the waste of shalgam juice process was revealed.

High-value-added residual components inside the waste are significant candidates as a potential to be used in different fields of industry both as a mixture or pure substance, if purification is performed. Further studies should be performed in order to optimize the extraction and detailed analysis can be performed to analyze the composition of phenolics, flavonoids and anthocyanins.

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The Declaration of Conflict of Interest

The authors declare that there are no competing financial and non-financial interest.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

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Farmers' Views on Organic Grape Production in Adıyaman Province: Method Adoption and Problems

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ABSTRACT

Healthy life in a sustainable environment is possible with accessible food production. The Covid-19 epidemic is a serious threat worldwide. This epidemic has revealed the importance of agricultural products. One of the most important areas of the epidemic affecting the whole world is exports; however, increased demand for agricultural products and exports in the epidemic process in Turkey. Turkey has a say in the trade of seedless dried grapes in the world. Seedless dried grape is on the healthy products list of the WHO. In 2019/2020, it had a share of 36% of world exports (with 246 thousand tons of export). This study was performed to evaluate their thoughts and farmers' problems with the adoption of organic farming in Besni district of Adıyaman. The research conducted in-depth interviews with the farmers producing organic grapes. Also, a face-to-face survey was conducted with 50 farmers. According to the findings, the land width of the farmers is not much. It has an average growing area of 37.52 decares. An average yield of 1.808 kg da^{-1} is obtained from this area. Serious differences have been found between conventional and organic farming. Moreover, government supports, high product prices, income, health, sustainability, and environmental protection are reasons farmers switch to organic farming. However, farmers argue that organic farming is less costly than conventional farming. Access to chemicals and marketing are major problems. As a result, farmers should be informed about organic farming. Additionally, it is thought that these studies will increase the productivity and product quality of the farmers. It is predicted that it will prevent rural to urban migration in the region.

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Introduction

Agricultural activities are among the current topics in every period. Agriculture is indispensable in terms of its contribution to community nutrition (Kaya, 2021) modern methods are important in agricultural production. New varieties and appropriate input use are required. It is desired to ensure production efficiency and a higher yield per unit area. The implemented methods should ensure agricultural sustainability. In addition, it is necessary to protect the environment and human health. This view increases the tendency towards organic farming and organic products.

Organic farming is known as environmentally friendly in respect of agricultural sustainability. It preserves the ecological balance as it is applied within certain rules (Kaya, 2021). It is an ecologically intensive production system expanding worldwide as the demand for sustainability increases (Eyhorn et al., 2019). It is an agriculture method that aims to evaluate the future of humans and the ecosystem as an indivisible whole with healthy plant and animal production (Akkurt et al., 2018; Gülgör Doğan, 2017). It is an integrated agricultural system that is environmentally, socially, and economically sustainable (Lampkin, 1990; Willer et al., 2019). Farmers depend on reliable provisioning of yields, profits, and environmental services to ensure production system sustainability over time. Moreover, reduced yield variability is necessary to ensure reliable food access for consumers (Schmidhuber and Tubiello, 2007; Müller et al., 2018; Mehrabi and Ramankutty, 2019).

Today, there are serious increases in the growing area and production amount of organic products. Organic farming method is applied for many products in Turkey (Kaya, 2021). Organic farming in Turkey started in line with demands from EU countries in 1985. It only started with traditional products such as dried grapes and dried figs, in the early years. Today, it has reached a sectoral status with more than 200 products that can be classified as herbal products, processed food, and other organic products (Öztürk and Islam, 2014; Kaya and Bay, 2020). According to the report published by IFOAM and FIBL

Research Institute, the economic magnitude of the organic market globally is around $81.6 billion. Whereas the organic market share among the EU countries is around $27.1 billion. This would mean that one third of the global organic market network belonged to the EU countries (Merdan, 2019). The demand for organic food is especially concentrated in Western Europe and North America. Most of this supply is provided by developing countries. Organic product trade is possible with compliance with legal regulations and international standards. Due to its geographical proximity and strategic, Turkey should expand its share in the EU market for organic food trade (Gök, 2008). Organic production is most intense in Ireland, Italy, and Romania, respectively. On the other hand, Germany, the Netherlands, Italy, and France are among the countries that import organic products, while the countries that export the most organic products are Germany, Italy, and Poland. In addition, the findings of this study demonstrate that Italy is the country that adopted organic farming in the fastest and most organized way among the EU countries (Merdan, 2019).

The number of farmers engaged in organic farming in Turkey has been higher than 80.000 in recent years (Kaya and Bay, 2020). Consumer demand for organic farming and food products has naturally increased the number of farmers who adopt organic farming (Demiryürek, 2011). The number of farmers involved in organic crop production was 53,782 in 2019. In addition, there are 170 farmers engaged in organic animal production in Turkey. Organic farming also has an important role in world trade. Turkey has an organic product export value of over $ 200 million in 2019 (Kaya, 2021). However, Turkey's possible to be processed into products for the world market. Turkey has a competitive advantage. It has advantages such as geographical location, climate characteristics, product variety, soil quality, and a high labor force working in agriculture. It is necessary to increase the market share, raise the awareness of the consumer, and encourage the consumption of organic products. In addition, farmers should be provided with access to information sources and trained (Özbağ, 2010; Rehber, 2011). The attitudes of people demanding organic products must be measured and determined. Consumers using organic products and high-income consumers have more positive opinions about organic products. There are health factors, environmental protection factors, innovation, agricultural support, and economic factors (Kurnaz, 2020; Kaya and Bay, 2020; El Bilali, 2020).

There are differences between traditional and organic products, such as production costs, cost items, and profitability levels (Yercan, 2003). Traditional farmers want to switch to organic farming due to agricultural support. However, the loss of yield and lack of knowledge in organic farming negatively affect the transition to the method. Organic farmers have problems with the amount of support, marketing opportunities and access to technical information (Karabaş and Gürler, 2011). In order to achieve the desired growth in the sector, it is necessary to increase the farmer/consumer awareness level of organic farming. There is a need for agricultural policies that meet national/regional needs (Baysel, 2013). In Turkey, consumer awareness of organic products is also low (Özbağ, 2010). Turkey's share in the world organic farming sector is important in terms of both production and consumption (product type, production amount, export revenues and consumption amount) (Vatansever Deviren and Çelik, 2017). However, it is observed that the farmers are experiencing significant problems and the expansion is progressing slowly. In this context, sales and marketing emerged as the most important problem. In addition, disease and pest control are other important problems in production (Kızılaslan and Taner, 2011).

According to FAO, approximately 77.1 million tons of grapes were produced in 7.7 million hectares of area in the world in 2019. Turkey is the world's largest seedless dried grape producer and exporter. Approximately 60% of the grapes produced in Turkey are with seed, according to the Turkish Grain Board of Agricultural Sector Report in 2019. In 2019, 4.1 million hectares of grapes were produced in Turkey. There was a total of 4.1 million tons of grape production, of which 2.050.000 tons for the table, 1.599.000 tons for dried (369.000 tons with seeds, 1.230 tons without seeds) and 451.000 tons for wine. In the production period of 2020, grape production was realized as 4.2 million tons. Approximately 70-75% of seedless dried grapes produced all over the world are subject to international trade. The remaining part is consumed in the domestic markets of the producer countries. The domestic consumption of the producer countries is around 250-350 thousand tons. Turkey's seedless dried domestic grape consumption is 35-50 thousand tons. Dried grape is a product that can take a bigger share of the world's organic food market in the future (MAF, 2021). In organic grape cultivation, the provinces of the GAP region are important organic farming basins. The total organic fresh grape production amount of the GAP Region is 22.281,09 tons. Dried grape production amount is around 4.801,60 tons. A total of 27.082,69 tons of organic grapes are produced in GAP provinces. This production is 16,21% of the total organic crop in Turkey. There are 26 different organically produced products in the region. Among these products, besides the grape table production, it has begun to be marketed by transforming it into value-added products such as dried grape, grape juice, molasses, fruit pulp, grape sausage and cutting. Organic grape cultivation is carried out on 22.388 hectares in Turkey. Organic production is 13.961 ha, and 8.427 ha is in transition. According to the data from 2017, a total of 228.432,50 tons of organic grapes are produced in this area, of which 91.838,79 tons are organic and 136.593,71 tons are in the transition phase. A total of 1.285,30 tons of organic grapes, including 1.276,30 tons of fresh and 9 tons of dried grapes, were produced in Adıyaman in 2017. In the transition period, there is a total of 11.452,94 tons of grapes, 7.696,32 tons of fresh and 3.756,62 tons of dried grapes (Özdemir et al., 2019). Despite lower yields and greater yield variability, organic methods had similar costs to conventional methods and were more profitable due to organic premiums (Smith et al., 2019). In spite of lower yields and greater yield variability on organic farms, organic farms were more profitable, and had similar costs compared to conventional farms. This is likely due to the organic premiums received, which can vary with market conditions and mitigate the effects of lower yields (Crowder and Reganold, 2015). Farmers should be encouraged to do organic farming (Kızılaslan and Olgun, 2012).

This study was carried out in order to reveal the attitude, problems and views of grapes producing farms towards organic farming and innovations in Turkey.

Materials and Method

The main material of the research is the data obtained from organic grape farmers. The study was carried out in Adıyaman, one of the most important grape producers of the Southeastern Anatolia Region, in 2017. It was examined as an in-depth interview and survey. The farmer lists of the Besni District Directorate of Agriculture were used to collect the necessary data. Farmers who received organic farming support and engaged in grape growing were determined. A survey was conducted with the full count method according to these lists. This study was conducted with 50 certified organic grape farmers. In the study, the minimum number of farmers could not be reached, as the farmers is not in the farms Modern methods are important in terms of the efficient use of natural resources and ensuring sustainability in agriculture. The province of Adıyaman in the Southeastern Anatolia Region has great importance in this regard. The improvement of irrigation possibilities with the dam has been effective in determining the location. It is also effective to increase the use of new technology and production methods. Southeastern Anatolia Region contributes to the agricultural potential of Adıyaman province. It is mostly in the foreground with field and vineyard-garden agriculture. In the last 20 years, important changes have occurred in the product pattern and production method of Besni.

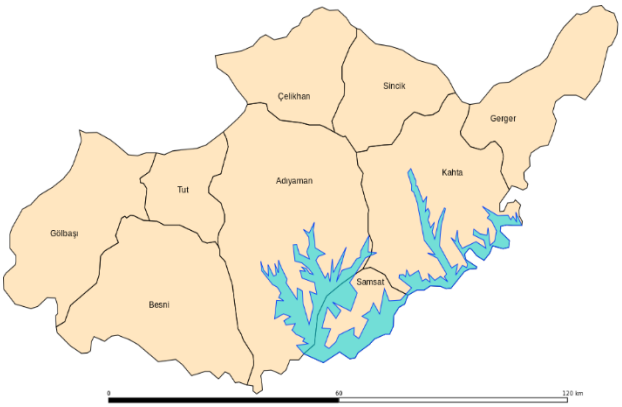

Figure 1. Location of the study (Anonymous, 2021)

The survey forms prepared were pre-tested. Data were collected after necessary arrangements. Field observations and group interviews were also used to develop and support the data set. The methods to be used in data evaluation were selected in accordance with the aims of the research. The analyzes were evaluated using the SPSS package program. The views of the farmers and the sources of information they use were analyzed with a Likert scale. The reliability of variables was measured with Cronbach's Alpha coefficient. In the SPSS program, reliability analysis is determined by the importance of the Cronbach alpha coefficient and the within-group correlation coefficient. The analysis is reliable when Cronbach's Alpha coefficient is between 0.60 and 0.80. This method is the weighted standard average of change (Özdamar, 1999; Kalaycı, 2016).

Results and Discussion

There were findings obtained as a result of face-to-face interviews with the farmers who voluntarily participated in the study. The average age of the farmers is 52, and more than 80% of them have received a primary school education. They are experienced individuals and consist of families with an average of 5 people. In addition, 70% of the farmers have social security. More than 40% of the farmers also have agricultural insurance in order to guarantee their agricultural products. It was determined that farmers' age average is high and their education level is low.

Land Use and Agricultural Activities

Most of the farmers in the region grow products such as grapes, pistachio, almond, wheat, barley and pepper. 56% of the farmers had a planting area of 30 decares or less. 20% of them had between 31-60 decares and 24% over 60 decares (Table 1). Merdan (2019) it was reported that according to data belonging to 2016, organic farming areas globally constituted 1% of the total agricultural area, whereas 6.7% of all the agricultural area was utilized as organic farming land in the EU.

Grape growing area of the farmers was between 4-128 decares. As seen in Table 2, the growing area was approximately 37.52 decares. The growing area of organic grape, which can be marketed as fresh and dried, was not very wide. In addition, it was determined that the yield of fresh grapes was low. Dried grape yield had an average value. Different grape varieties are also grown in the region.

The high production costs in the region were a challenge for farmers. Even if the cost of chemical pesticides was not much, labor and diesel cost were very high. Only sulfur was applied for plant protection in the region. The amount of inputs used by farmers varied according to the land size. Labor, which constituted the most basic cost, was determined as 61%, diesel 32% and protective products 7%. The state supports used in this context relieved the farmers a little. All of the farmers benefited from organic farming support. However, approximately 60% of the farmers found organic farming support insufficient. There were also farmers who benefited from diesel, fertilizer, animal husbandry and feed support. In addition, approximately 60% of the farmers had animal assets.

Regarding organic farming, 22% of the grape farmers were affected by the village headmen. Farmers usually stated that they did not know enough about the subject. In the study, nearly half of the farmers (51%) find medium level knowledge of organic farming. In addition, 30.6% of the farmers think they are knowledgeable about the method. Acıbuca et al. (2018) it was said that for the farmers to whom the survey was applied, the most significant problems observed are marketing, absence of contract production, the used pesticides' being ineffective and the lack of information on growing methods. It is thought that farmers have a low income because they keep doing organic farming in order to benefit from agricultural support in spite of having significant problems; they do not have any production methods and the ability to cope with diseases and pests. That they are not aware of the possibility of benefiting from the information acquisition sources when a single problem is faced, makes the need for raising the dissemination activities for farmers.

Table 1. Distribution of farmers by grape cultivation area (%)

Grape growing area (da)	Frequency	%
30>	28	56
31-60	10	20
60<	12	24
Total	50	100

Table 2. Farmers' average values for grape production

Area (da)	Yield (kg da^{-1})	Price (₺ kg^{-1})	Type (da)		
			Property	Rent	Sharecropper
37.52	1808.40	7.46	40.83	37.67	-

Table 3. Farmers' knowledge level regarding good agricultural practices

Good agricultural practices	Frequency	%
Be informed	14	28.0
Not be informed	36	72.0
Total	50	100.0
Knowledge level	Frequency	%
Little	7	14.0
Middle	5	10.0
Very	2	4.0
Declarative opinion	14	28.0
Not declarative opinion	36	72.0
Total	50	100.0

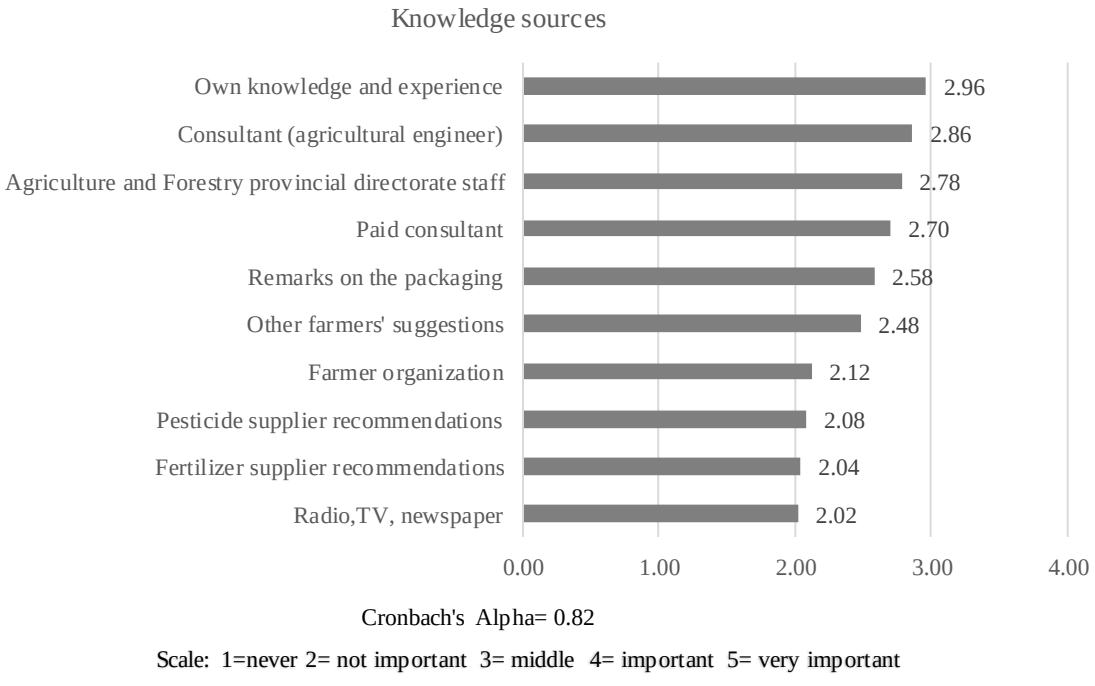

Figure 2. Knowledge sources used in dose adjustment in agricultural inputs

Farmers' Reasons for Adopting Organic Farming

Many factors affect the transition to organic farming for this region, as in the world. These factors are generally encountered in terms of economic as well as environmental and social. The study determined that farmers benefited from organic farming support, which was one of the important reasons for the transition to organic farming. Health, environmental awareness and income growth follow, respectively. In addition, gaining experience in organic farming, having a certificate and setting an example for other farmers are among the reasons that affect the transition to organic farming. İpek and Yaşar Çil (2010) it was stated that with the increase in global pollution, a number of regulations are made for organic farming at both international and national levels. Acıbuca et al. (2018); Karabaş and Gürler (2011) it was determined that the low agricultural incomes of the farmers are also effective in the development of organic farming activities in the region. In addition, the absence of control and certification organizations and companies marketing organic products limits the sources of information for farmers.

Farmers' views regarding good agricultural practices

Good agricultural practices (GAP) are also important for human health and the environment. It is a different growing method like conventional farming and organic farming. However, the farmers in the region did not know much about the GAP. Approximately 2/3 of the farmers stated that they did not have information the GAP. In addition, as seen in Table 3, the knowledge level of the farmers on good agriculture practices was low and medium. Most farmers did not comment on the GAP because they did not have sufficient knowledge (Table 3).

Knowledge Sources Used in Dose Adjustment in Agricultural Inputs

Farmers act according to different knowledge sources while adjusting the dose of agricultural inputs. They usually rely on their own knowledge and experience of the past years. Other knowledge sources are given according to the level of importance in Figure 2.

Farmers' views on Organic Farming

The views of the farmers on organic farming are given in Table 4. It was understood that organic farming contributes significantly to the region's economy. It was also an advantage that the products were generally demanded by all consumers. Moreover, it prevented migration from rural to urban (Table 4). Kahveci and Ataseven, (2020) it was said that farmers engaged in organic farming come together with organic markets and other organizations. Thus, they both increase their income and offer solutions to consumers' wishes. Organic farming has some fundamental problems that limit development in Turkey. The most important of these problems is that farmers engaged in organic farming cannot be organized. Müller et al. (2018) it was determined that low variability allows farmers to achieve consistent production and avoid unprofitable years while ensuring that consumers have reliable access to nutritious and sufficient food. When farmers are able to generate consistent crop yields, food prices are also less volatile and global trade markets are more stable.

Problems Encountered in Grape Growing

There are problems faced by farmers in grape growing. The most important problems are labor, lack of sufficient markets and buyers, low product prices and insufficient state support (Table 5). Karabaş and Gürler (2011) it was stated that there are many factors in the fact that conventional farmers do not want organic farming. These factors are the loss of yield in organic farming, lack of knowledge about organic farming and not making market oriented production. Organic farming producers were determined to have problems regarding the lack of incentives and market place. In addition, there are problems accessing technical information about organic farming.

Low cooperation and organization among farmers is also an important problem. The lack of membership of the farmers in any union or cooperative increases the problems. As a result of the study, it was determined that 90% of the farmers do not have such membership. Others were members of the Chamber of Agriculture, the Agricultural Credit Cooperative and the grape farmers' association they have established with their local means. In addition, farmers had a marketing problem. Due to poor organization, they sold their products at a lower price. For this reason, 34% of the farmers sold to the merchants who come to their villages. In addition, 44% of them found the opportunity to sell to brokers and 22% to the public market (Figure 3). Karabaş and Gürler, (2011); Peeters et al., (2020) it was said that organic farming support should be increased, and farmers should be supported in providing market places for local governments.

Table 4. Farmers' views regarding organic farming

Views	Means	Cronbach's Alpha
It contributes to the economy of the region	2.94	0.73
Demanded by all consumers	2.80	
Organic farming prevents rural-urban migration	2.78	
Organic farming farmers have higher incomes	2.66	
Input prices are high	2.56	
There is a problem of fighting diseases and pests	2.48	
Organic farming is more costly than conventional farming	2.42	
Organic products have a marketing problem	2.40	
Difficult to access pesticides used in organic farming	2.34	
Organization and cooperation between farmers is insufficient	2.32	
The region is not suitable for organic farming	2.04	
There is no difference between organic products and traditional products	1.64	

Scale= 1=Not agree; 2=Partly agree; 3=Agree

Table 5. Farmers' views on problems encountered in the grape growing

Problems	%
Not finding sufficient markets and buyers	88
Water supply and irrigation problem	54
Low product prices	88
Disease and pest control	80
Labor problem	90
Lack of government support	76
Finding suitable regions for organic farming	30

*More than one option is specified (%).

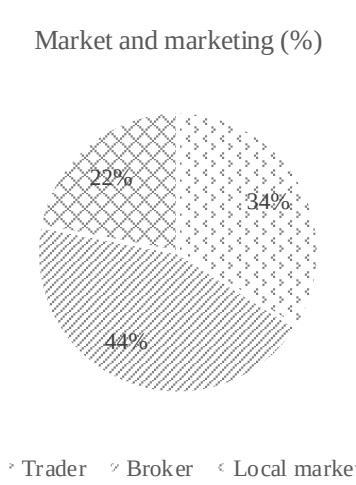

Figure 3. The way farmers' market their products (%)

Demiryürek, (2011) it was determined that most of the current organic production is exported mainly to the EU countries and the domestic market has been growing. Though, Turkey has suitable ecologic conditions and export potential for organic production, the share of Turkish organic products in the global market is significantly low.

Conclusion

Vineyard, pistachio, fruit growing, almond and olive cultivation are common in the region. The soil structure of the region and suitable climatic conditions allow it to grow more than one crop. Local farmers should follow all financial support and opportunities to improve organic production. The region's people should be raised awareness of the importance of natural resources. The low level education of farmers affects their adoption of innovations. Farmers do not have enough information about organic farming. It has been determined that those who know about organic farming are also lacking in scientific terms. Farmers acquired the knowledge mostly from relatives, leading farmers and courses. Farmers must be informed about the environment and health. The use of information technologies in agriculture should be increased. Computer and internet usage should be expanded. Training programs should be organized regularly in terms of spreading innovations. Agricultural extension activities should be increased. All of the farmers benefit from organic farming support. However, approximately 60% of the farmers find this support insufficient. Agricultural and environmental sustainability should be supported. Market and marketing, low product price, and water and irrigation problems are among the most important problems. The organizational status should be strengthened by increasing cooperation between farmers. Products should be marketed with added value. There is a need for a packaging facility in this area. Domestic and foreign organic markets should be expanded. Farmers should open up to international markets as well as government subsidies. It should not be forgotten that all studies on this issue will greatly contribution to the global economy, primarily the regional economy.

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Consumer's Perspectives on Misinformation Links with the Consumption of Broiler Meat: A Case of Kandy District - Sri Lanka[#]

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ABSTRACT

The study described herein aimed to investigate the relationship between perceptions of hormone usage and customer preferences for broiler meat and meat products in Sri Lanka with special reference to Kandy district. A total of 460 respondents from Kandy district were interviewed using a pre-tested questionnaire. The analysis revealed that 85.9% of the respondents believes the fact that the hormones are used in broiler production. Also, 75.7% of the respondents were unaware about the fact that the hormones are totally banned from Sri Lankan broiler production. Around 71.4% believed that the hormones are still being used illegally in broiler production in Sri Lanka. The study also found that the general public (36.2%), was the main source that the respondents perceived this false information concerning hormone use. Similarly, 83.7% believes that these chemical substances create health hazards to human. 76.7% of the respondents strongly believed the fact that the adolescent girls who consume broiler meat regularly during their childhood may experience early puberty. The findings of the present study concluded that three misconceptions of (i) use of hormones to attain high growth rates in broilers (ii) hormones assumed to be present in broiler meat pose health hazards to public and (iii) frequent broiler meat consumption during childhood is accompanying with the early puberty in adolescent girls, do exists. Though the majority of the sample comprises of highly educated professionals, these misinformation were spreaded from the information generated among the general public. However stipulating a valid certification with no added hormone in broiler chicken meat will be helpful in changing the mind-set of general public.

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Introduction

Broiler meat is one of the most crucial animal originated protein sources which can contribute to nutritious, healthy and balanced diets at an affordable price (Fiala, 2008). As a consequence, the global broiler chicken production has skyrocketed to meet both the global demand and the financial gain (Najeeb et al., 2014). Broiler chickens (*Gallus domesticus*) are bread primarily for their meat and are popular due to their faster growth rate, minimal fat cover, tender and delicate flesh (Haque et al., 2020). Moreover, the current broiler sector has a competitive advantage of having a better feed conversion efficiency than other food animals, use fewer inputs and expenses and provide a greater output per unit area (Ozturk et al., 2012; Ozturk 2017; FAO 2022). Furthermore, chicken meat is crucial in ensuring a sustainable food supply since it emits fewer greenhouse gases than other protein sources (Caro et al., 2017).

Poultry meat is the most rapidly expanding meat category in Sri Lanka (Prabakaran, 2003). Over the last three decades, poultry industry, remarkably the broiler industry in Sri Lanka, has been progressed from backyard system to a highly commercial industry with the strong and passionate participation of the private sector. Sri Lanka has 12,583 broiler farms and 83,611 poultry enterprises in 2020, where 216.16 (000 MT) of chicken meat and 11,220.40 (MT) of chicken meat-based products were produced (Livestock statistical bulletin, 2020). As revealed, the annual per capita chicken consumption in Sri Lanka is 9.79 kg, whereas the world average is 8.57 kg (Livestock statistical bulletin, 2020).

Before evolving new technologies in the poultry industry, it took around 120 days for a broiler to reach a weight of 1.5 kg; currently, it is accomplished only in 30 days. Thus indisputably, the broilers are characterised by their faster growth over a short period (Jaturasitha et al.,

2017). Furthermore, at the end of 6 to 7 weeks of production period, the modern broiler industry can produce broilers weighing around 2 kg or more (Maurer, 2003). Genetic improvement and improved nutrition have contributed to the large size broilers. Moreover, the general productivity benefited from the pleasant environment and the appropriate management practices have identified as best contributed to the bird's productivity.

However, striking changes that have occurred over the time might make people to concern about the rapid growth rate of broilers. Therefore, the use of hormones in broiler meat production has recently received a notable attention from the sides of consumers, media, and other entities. It has been traced that six hormones had been licensed and approved by the Food and Drug Administration (FDA, 2016) to be used in livestock production. Several hormones such as progesterone, 17-β-oestradiol, testosterone and its anabolic derivatives like zeranol and clenbuterol are commonly used in livestock production to fatten the animals (Hirpessa et al., 2020). Oestradiol, testosterone and progesterone are the natural sex hormones therefore, meat cannot be tested for the presence of these hormone residues since the animals produce them naturally. Zeranol, trenbolone acetate and melengestrol acetate are growth-promoting synthetic hormones and in human, these hormones have been proven to be mutagenic, genotoxic, teratogenic, neurotoxic, and carcinogenic (Gandhi and Snedeker, 2000; Hirpessa et al., 2020). Though hormones assist the feedlot cattle in gaining weight and increasing feed efficiency before slaughtering, regulations do not permit hormone to be used in poultry (ducks, chickens and turkeys) or pork production (USFDA, 2015; Yang et al., 2017). It is noteworthy that international legislation has been enacted restricting the usage of hormones in poultry (Hirpessa et al., 2020). The use of hormones (anabolic steroids such as oestradiol, testosterone, nandrolone, stilbene, estrogen, progestogens etc.) for food animals is banned in Sri Lanka (Gazette No. 1,292, 06.06.2003). Though it has never been utilised in poultry in several countries, in 1970's, the use of hormones in poultry diets was forbidden in some countries (FDA, 2016).

Although consumers may have a different perspectives on the use of hormones in livestock production, misinformation added by the media may mislead the consumers to assume that the meat industry often uses hormones to fatten the animals (Yang et al., 2017). However, social media platforms highlight the false belief that "chickens are given hormones to make more meat" has spreaded worldwide (Esquivel-Hernandez et al., 2016). This misinformation imposed a negative repercussion for the poultry sector whilst establishing false public health concerns such as cancer, obesity and early puberty in adolescent females though there are no added hormones available in commercial broiler chickens (Esquivel-Hernandez et al., 2016). Moreover, faulty food labels also creates doubts and influences the perceptions of the quality of the product (Yang et al., 2020). Consumers can find poultry products those are branded as 'No added hormones' in supermarkets, even though hormones are not used during the production chain. This may lead consumers to believe that hormones are used in poultry production (Yang et al., 2020).

Antibiotics have been a standard part of the broiler diet for decades. Antibiotics were incorporated in broiler diets keeping the prime objective as disease prophylaxis, control and treatment, and as a growth promoter to enhance productivity and feed efficacy (Tollefson and Miller, 2000; Gaskins et al., 2006). However in 2006, the use of antibiotics in poultry diets were banned in many countries (FDA, 2016) while Sri Lanka, Indonesia, Bhutan Bangladesh, Myanmar, Thailand and Nepal imposed antibiotic restriction (Cardinal et al., 2019). However antibiotic residues, antimicrobial resistance, pesticide residues, are of wider concerns in the current broiler industry (Haque et al., 2020).

According to Clark et al. (2017) and del Bosque et al. (2021), purchasing decisions of consumers for broiler meat are influenced by many factors. Price is unquestionably one of the major factors considering when purchasing meat. Nonetheless, understanding purchasing decisions for varied meat products is also more or less driven by perceptions held by consumers. Since consumer beliefs influence consumer decisions, monitoring consumer beliefs is noteworthy for assessments of consumer behaviour (Lusk et al., 2014). Researchers from different countries evaluated the consumer concerns about hormone usage in broiler meat (Gandhi and Snedeker, 2000; Yang et al., 2020). However, only very limited number of systematic studies were carried out in Sri Lanka to evaluate the public's knowledge on hormone-free broiler chickens. Therefore, this work aimed to investigate the relationship between perceptions of hormone usage and customer preferences for broiler meat and meat products in Sri Lanka with specification to Kandy district.

Materials and methods

The present study used a deductive approach utilizing both primary and secondary data. The primary data collection was obtained through a pre-tested, interviewer-administered questionnaire and field observations. The secondary data was gathered from the Department of Census and Statistics, the Ministry of Livestock and Rural Community Development and the reports from the Central Bank. The questionnaire was developed to assess consumer perception, purchasing behavior, and consumption patterns concerning broiler chicken meat. In sampling, the number of questionnaires was decided based on the district's population. A total of 460 respondents from Kandy district were selected using a simple random sampling method. Kandy district was deliberately chosen for the study because (i) of having a higher number of poultry farms (11,156), (ii) it ranks the fifth and eleventh in terms of the overall number of poultry farms and broiler farms in Sri Lanka, respectively, and (iii) as a central provincial district where the poultry industry is dominant (Alahakoon et al., 2016; Census and Statistics, 2020). Collected data were recorded and were processed in a database formed in Statistical Package for Social Sciences (SPSS - version 22) and Microsoft Excel 2016 software. The data were analyzed using descriptive statistics, including frequency analysis, and Pearson's chi-squared test was used to determine the relationship between the variables obtained.

Results and discussion

Socio-economic background of the respondents

According to the findings, the majority of the respondents were male (52.6%) and were unmarried (55.4%) (Table 1). The most common age group participated in the survey ranged between 18 and 30 years (53.9%). Moreover, 45.4% of the respondents were urban dwellers, whereas 54.6% were rural dwellers. The majority of the responders (48.9%) have completed their higher education (graduated or are currently enrolled in postgraduate programs), whereas 46.3% of the sample have completed their secondary education. Most respondents stated their primary occupation as "other" (41.1%) and earn between Rs. 25,000 and Rs. 50,000 per season/month.

Response to the questionnaire

The results indicated that 85.4% of the total respondents consume broiler meat. Those who do not prefer broiler meat (14.6%) were given with the reasons (i) unpalatability (41.9%), (ii) concerns about how the broilers are raised (24.7%) and (iii) the religious beliefs (18.3%) as reasons for their rejection (Figure 2).

According to the study conducted in the southern province of Sri Lanka (de Silva et al., 2010), the respondents claimed the religious beliefs (74%), economic concerns (47%) and antipathy toward killing animals (82%) as reasons for refrain from eating broiler meat. Similarly, several other authors have found that the religious views have a significant impact on meat consumption patterns (Delener, 1994; Pettinger et al., 2019). However, the current study revealed that the religious beliefs (18.3%) play a minor role in not being a broiler chicken meat consumer, while economic concerns are also least affected or almost (0%) zero when compared to de Silva et al. (2010). However, it is obvious that the percentage of participants in the present study who do not consume chicken due to their religious beliefs has declined. Thus, it seems that the attitudes of the consumers have shifted over the time, positively for alleviating malnutrition in the country.

In spite of reputation of broiler meat as healthy white meat, the chicken was more popular among females than males (de Silva et al., 2010). However, in contrast, the current study revealed that the males (89.3%) are more interesting on consuming chicken meat than the females (81.2%). Furthermore, this will express that the attitude and preference of the community can be changed time to time.

The correlations between the consumption of broiler chicken meat with other sample variables like gender, age, and income are presented in Tables 2, 3 and 4, respectively. The Chi-square tests indicated a statistically significant positive relationship between gender and chicken consumption ($p=0.014$), thereby rejecting the null hypothesis (Table 2). However, there is no significant correlation between chicken consumption and income level ($p=0.141$) with the factors such as education and occupation.

Table 1. Socio-economic background of the respondents

Characteristics	Frequency	Percent (%)
Gender		
Male	242	52.6
Female	218	47.4
Age		
Below 18 years	20	4.3
Between 18 – 30 years	248	53.9
Between 30 – 60 years	137	29.8
Over 60 years	55	12.0
Locality		
Urban	209	45.4
Rural	251	54.6
Marital status		
Married	205	44.6
Unmarried	255	55.4
Highest education level		
Primary education	22	4.8
Secondary education	213	46.3
Higher education	225	48.9
Working sector		
Government	86	18.7
Private	139	30.2
Self-employment	46	10.0
Other (include jobless)	189	41.1
Income level (Rs.)*		
Below 25,000	152	33.0
Between 25,000 – 50,000	155	33.7
Between 50,000 – 100,000	107	23.7
Over 100,000	46	10.0

* Sri Lankan Rupees (LKR); Source: Field Survey from November 2021 to January 2022.

Table 2. Chi-square test results: Gender and consumption of broiler meat

Gender × Broiler meat consumption	Value	Degree of freedom	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson Chi-Square	5.993[a]	1	0.014		
Continuity Correction[b]	5.362	1	0.021		
Likelihood Ratio	6.011	1	0.014		
Fisher's Exact Test				0.017	0.010
Linear-by-Linear Association	5.980	1	0.014		
Number of Valid Cases	460				

[a]Chi-square test results (Gender Vs. broiler meat consumption) 0 cells (0.0%) have expected count less than 5. The minimum expected count is 37.75; [b]Computed only for a 2x2 table; Source: Field Survey from November 2021 to January 2022.

Table 3. Chi-square test results: Age and broiler meat consumption

Age × Do you consume broiler meat?	Value	Degree of freedom	Asymptotic significance (2-sided)
Pearson Chi-Square	10.713[a]	3	0.013
Likelihood Ratio	9.741	3	0.021
Linear-by-Linear Association	9.736	1	0.002
Number of Valid Cases	460		

[a]1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.91.; Source: Field Survey from November 2021 to January 2022.

Table 4. Chi-square test results: Income level and broiler meat consumption

Income level × Consumption of broiler meat	Value	Degree of freedom	Asymptotic significance (2-sided)
Pearson Chi-Square	5.466[a]	3	0.141
Likelihood Ratio	6.623	3	0.085
Linear-by-Linear Association	1.126	1	0.289
Number of Valid Cases	460		

[a]0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.70.; Source: Field Survey from November 2021 to January 2022.

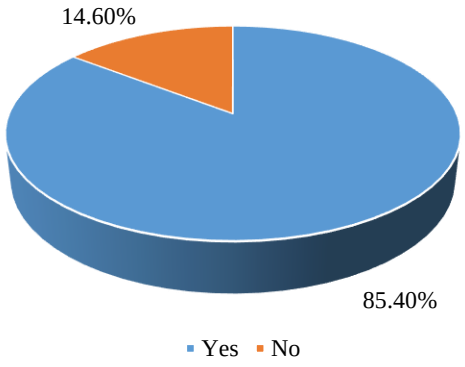

Figure 1. Preference for broiler meat consumption.
Source: Field Survey from November 2021 to January 2022.

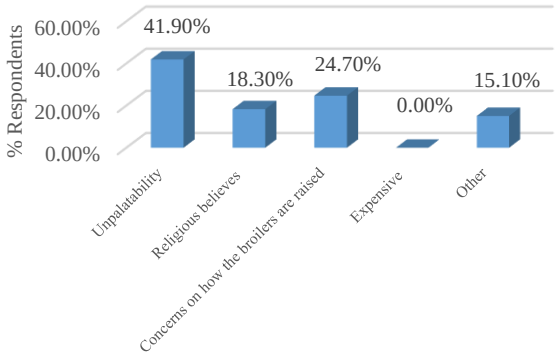

Figure 2. Reasons for not consuming broiler chicken meat.
Source: Field Survey from November 2021 to January 2022.

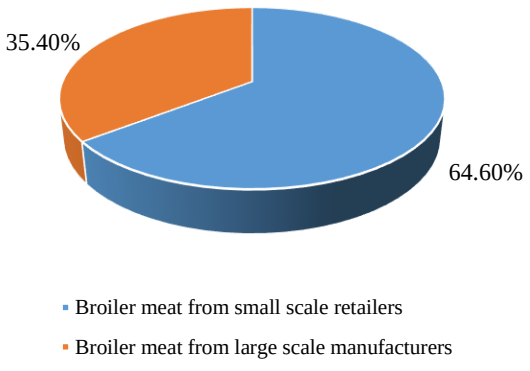

Figure 3. Broiler meat purchasing behavior.
Source: Field Survey from November 2021 to January 2022.

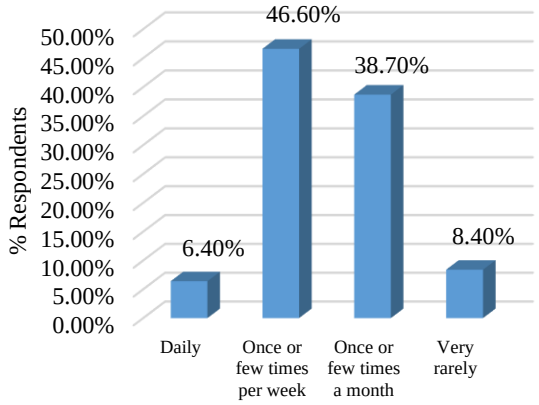

Figure 4. Frequency of consuming broiler meat.
Source: Field Survey from November 2021 to January 2022.

Table 5. Chi-square test results: Income level and frequency of consuming broiler meat

Income level × Broiler meat consumption frequency	Value	Degree of freedom	Asymptotic significance (2-sided)
Pearson Chi-Square	38.935[a]	9	0.000
Likelihood Ratio	44.967	9	0.000
Linear-by-Linear Association	16.010	1	0.000
Number of Valid Cases	393		

[a]2 cells (12.5%) have expected count less than 5. The minimum expected count is 2.80.; Source: Field Survey from November 2021 to January 2022.

Table 6. Chi-square test results: Highest education level and knowledge about broilers length of production

The highest educational level x consumer knowledge about broiler chicken reaches their market weight within a short period	Value	Degree of freedom	Asymptotic Significance (2-sided)
Pearson Chi-Square	33.788[a]	2	0.000
Likelihood Ratio	31.996	2	0.000
Linear-by-Linear Association	31.106	1	0.000
Number of Valid Cases	393		

[a]0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.98.; Source: Field Survey from November 2021 to January 2022.

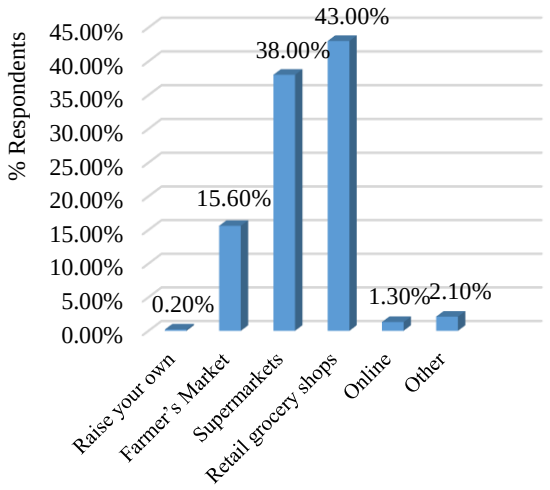

Figure 5. The purchasing behavior of broiler meat.
Source: Field Survey from November 2021 to January 2022.

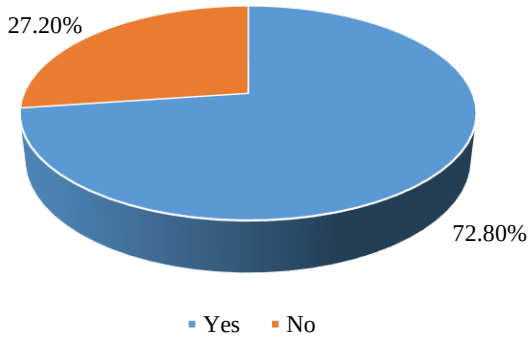

Figure 6. Awareness on production length of broilers.
Source: Field Survey from November 2021 to January 2022.

This is in a complete agreement with the findings of de Silva et al. (2010), as gender was found to have a significant effect on meat consumption while no significant correlation exists between meat consumption and educational level. Nevertheless, significant correlations exist between the chicken consumption and age (Table 3). Moreover, having an inverse relationship, it was proven that with the age, they become more health conscious and thus reduce their meat consumption (He et al., 2003; de Silva et al., 2010).

The purchase behavior focuses primarily on customer preferences for spending their resources on meat-related items (de Silva et al., 2010). The meat purchasing behavior indicated that the majority of the respondents purchase broiler meat from the small-scale retailers (64.6%), while the rest (35.4%) purchase broiler meat from the large-scale manufacturers (Figure 3).

According to Figure 4, nearly 46.60% of the participants consume broiler meat once or a few times a week. This is followed by a frequency of once or a few times a month (38.70%). The least number of respondents consume either broiler meat daily (6.40%) or very rarely (8.40%). However, studies conducted in Turkey and Finland revealed that the majority of the society consumes broiler meat for a frequency of once or twice a week which implies that all three countries have relatively the same consumption frequencies (Pouta et al., 2010; Durmuş et al., 2012).

Moreover, the chi-square analysis indicates a statistically significant positive relationship (p=0.000012) between the income level and the consumption frequency (Table 5). This is in an agreement with Putnam and Gerrior (1997), who found that the purchasing power of meat and consumption habits are primarily determined by income, price, flavor, and preferences.

Most consumers purchase broiler meat from retail groceries (43.00%) and supermarkets (38.00%), whereas the retail groceries and supermarkets are the dominant suppliers of the food supply chain in Sri Lanka. However, the others mentioned farmers' markets (15.60%) and online stores (1.30%) as the places where they purchase broiler meat.

Of the sample, 50.40% of the respondents had an idea on broiler production while the rest (49.50%) did not have.

Furthermore, only 72.80% of the total respondents were knowledgeable about the fact that the broiler chicken reach to their market weight within a short period, while 27.20% said that they did not aware of it (Figure 6). According to the chi-square analysis, there is a positive relationship between the educational level and consumer knowledge on broiler chickens' short production period (Table 6).

A significant quota of the sample (85.9%) believes that the hormones are used for broiler chicken at the production level, and a similar percentage (83.7%) believes that these substances pose health hazards to human (Figure 7). The study also found that the general public (36.2 %), followed by the print media (Newspapers, books and magazines etc.) (30.80%), were the sources that respondents perceived this false information concerning hormone use (Figure 8). Interestingly, the present study revealed that 75.7% of the respondents were unaware about the fact that the hormones such as anabolic steroids (i.e. Testosterone, progestogens, stilbene, oestradiol, and estrogen) are banned in Sri Lankan broiler production (Gazette No. 1,292, 06.06.2003), and 71.4% of them have stated that hormones are still being used illegally in broiler production in Sri Lanka. In another study conducted to assess consumer awareness on broiler nutrition with antibiotics and hormones in Istanbul, turkey, revealed that 88.3% of the consumers believed that the hormones are used in broiler chicken feeding, whereas 11.7% believed that it is not (Karasu and Ozturk, 2020). So both studies imply that the consumers have a generally poor understanding of the livestock industry, even though hormones are not permitted by law to be used in poultry.

However, Results from the Chi-square analysis prove that there is no correlation between respondents' level of education and their views on the use of hormones in broiler chicken production (p=0.732). Similarly, there is no correlation exists between the respondents' field of employment and their views on the use of growth hormones in broiler chicken production (Table 7). However, the current results do not agree with the results of the study conducted in Kars province in Turkey, as it demonstrated that the percentage of those who think that the chicken meat is risky in terms of hormones and antibiotics is also increased with the advancement of consumers' education level. And also, it reported that the news from the media is the most crucial factor triggering this decision (Ayvazoğlu Demir and Aydın, 2018).

Table 7. Chi-square test results: Profession and hormone use perception

Profession × Do you think that the hormones are used to gain a rapid growth rate in broilers?	Value	Degree of freedom	Asymptotic significance (2-sided)
Pearson Chi-Square	5.277[a]	3	0.153
Likelihood Ratio	5.899	3	0.117
Linear-by-Linear Association	4.277	1	0.039
Number of Valid Cases	460		

[a]0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.50; Source: Field Survey from November 2021 to January 2022.

Table 8. Chi-square test results: Highest education level and perception of frequent eating of broiler meat can cause early puberty in adolescent girls

The highest educational level × Do you think that eating broiler meat frequently during childhood leads to the earlier onset of puberty in adolescent girls?	Value	Degree of freedom	Asymptotic significance (2-sided)
Pearson Chi-Square	2.728[a]	2	0.256
Likelihood Ratio	2.530	2	0.282
Linear-by-Linear Association	0.053	1	0.818
Number of Valid Cases	460		

[a]0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.12.; Source: Field Survey from November 2021 to January 2022.

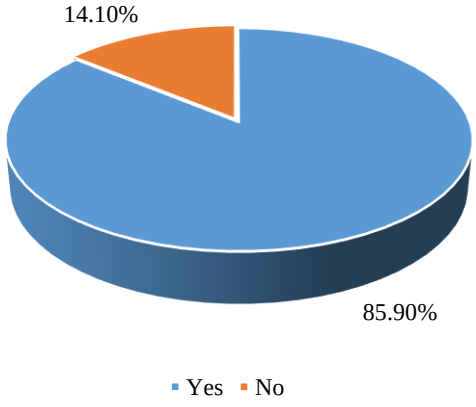

Figure 7. Perception on hormone use in broiler production.
Source: Field Survey from November 2021 to January 2022

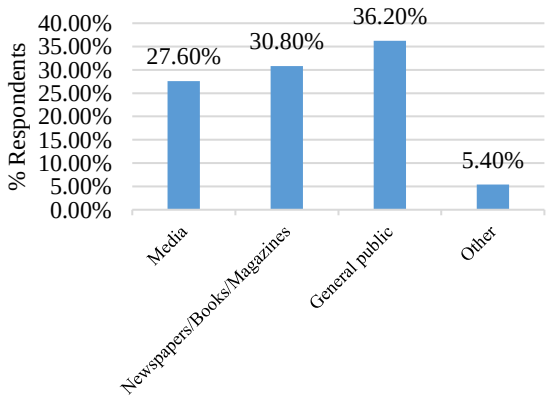

Figure 8. Sources that perceive misconceptions.
Source: Field Survey from November 2021 to January 2022.

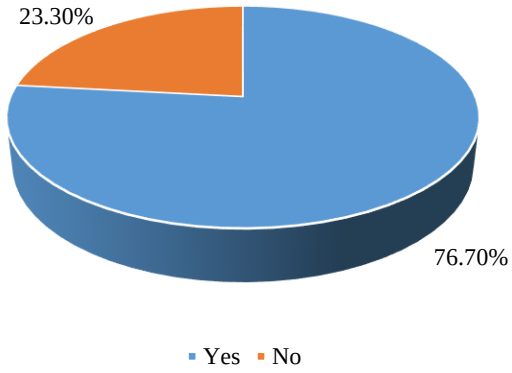

Figure 9. Perception on frequent broiler meat consumption can cause early onset of puberty in adolescent girls.
Source: Field Survey from November 2021 to January 2022.

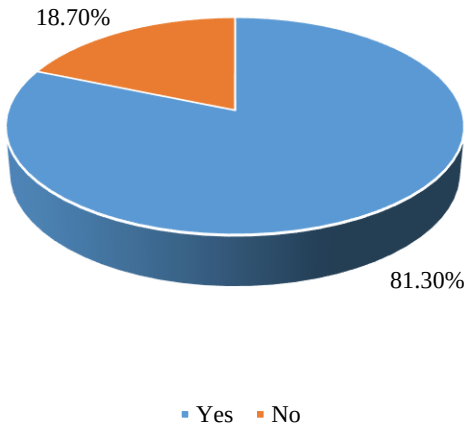

Figure 10. Attitudes of the consumers to change their mindset if the broilers are ensured and certified with ‘‘no added hormones’’ during the production.
Source: Field Survey from November 2021 to January 2022.

Furthermore, the respondents participated in the current survey (76.7%) highly agreed with the fact that the adolescent girls who consume broiler meat regularly during their childhood may experience early puberty (Fig. 9). According to the results of the current study, it has been revealed that the most of the respondents (42.10%) gathered this misinformation from the general public.

No correlation exists between the level of education and the notion that consuming broiler meat frequently can cause early puberty in adolescent girls (p=0.256) (Table 8).

Hormone usage in livestock production is controversial due to safety concerns. According to the research conducted in 1995 by the Food Marketing Institute, 50% of the consumers considered hormones as a severe health risk. Of the different consumer concerns related to broiler industry, the concerns on hormone usage and other growth boosters ranked the highest than the concerns on antibiotics, preservatives and additives but comparatively lower than the concerns on microbial spoilage and chemicals (Alahakoon et al., 2016). Moreover, some studies revealed that not all customers have the same confidence in the statistical claims made in the experiments, advertisements and surveys (Hayes et al.. 1995). However, Sri Lanka seemed to pose a different scenario, while the most of the people rely on information gather from the general public. However, the majority of the respondents (81.3%) agreed to change their mind-set if the relevant authority would ensure that the broiler chickens are certified with no added hormones during their production cycle (Figure 10).

Several studies demonstrated that there has been a significant increase in the demand for food transparency information, disclosing that the food labels have a significant impact on demand and customer purchases (Liaukonyte et al., 2013; Yang et al., 2020). Although, consumers depend on food labels to make informed choices about what they consume the rise of “antibiotic-free” and “hormone-free” labelled meat on supermarket shelves shows how labels can be both informative and misleading. Therefore, consumers’ perception of hormone usage impacts their selection of unlabelled meat products. Therefore, adopting these labels as a marketing tool confuses consumers and creates doubts about the meat safety. Thus, it implies that various factors, including safety guarantee, availability of exact information, quality assurance of the product, convenience and attention to animal welfare, play a significant role in determining customer satisfaction with meat and its products (Alahakoon et al., 2016). Reasonable efforts should be made to raise public awareness to dispel widespread myths and alter unfavourable perceptions of consumers about broiler chicken.

The sample size of the present study is representative of 1 of 25 districts in Sri Lanka. Therefore, generalization of the findings of this study should be approached with a care since the whole sample consists of customers from a single region called Kandy district. Perhaps, it may not reflect the perceptions of all Sri Lankans. In addition, it is essential to highlight that the focus of this study was on customer behaviour intentions rather than actual behaviour. Consequently, Future studies must examine a larger sample size that encompasses the entire island so that the results may be generalized to all Sri Lankan consumers.

Conclusions

This study concluded that the misconceptions of (i) use of hormones to acquire high growth rates in broilers, (ii) hormones assumed to be present in broiler meat pose health concerns to people and (iii) frequent consumption of broiler meat during childhood is associated with the early onset of puberty in adolescent girls, do exists. Though the majority of the sample comprises of highly educated professionals, these misinformation were spreaded from the information generated among the general public. Stipulating valid certification with no added hormone in broiler chicken meat will change the mind-set of general public. Therefore, uplifting the public awareness on legal background on hormone usage in food animals and accurate product labelling procedures are warranted.

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Determination of Production and Marketing Behaviors of Producers Selling Products to the Turkish Grain Board, (Kırşehir Province Mucur District Micro Field Study)

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ABSTRACT

The aim of this study is to examine the socio-economic characteristics of producers who sell wheat to the Turkish Grain Board (TGB), which is an interventionist organization in the purchase and sale of grain in Türkiye, as well as some of their behaviors regarding production, marketing preferences, and their relations with TGB. The sample of the study consisted of 100 wheat producers in the Mucur district of Kırşehir province. The obtained data were interpreted by converting them into tables, cross-tabulations, and graphs. The data in the cross tables were statistically interpreted using Chi-square analysis. It can be said that producers are not satisfied with TGBs' purchasing practices for some reasons. The most important reason for this is that quality-based purchasing practices have an extremely negative impact on prices. It was determined that they were not satisfied, and this resulted in high price reductions in quality-based purchasing, and as a result, there was distrust in analysis practices. Quality-based purchasing policy is a method that allows wheat quality characteristics to be determined with various devices in a short time. This method, which is decisive in quality classification and product pricing scale, must be explained correctly to producers. Otherwise, the producer 's trust in the TGB may gradually decrease. This can be achieved through initiatives taken by the institution or through various publications and training activities. One of the most important results of this study is that TGBs and decision-makers are more sensitive to local producers. For decision-makers to maintain their influence on the producer, they must carefully examine the details of the processes with an inductive approach, starting from the bottom up. This situation is considered critical in terms of food security.

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Introduction

Wheat is an indispensable food that forms the building block of human nutrition worldwide. Wheat; flour, pasta, semolina, biscuits etc. It is consumed as. In recent years, the demand for wheat has increased significantly because of the pandemic, the Russia-Ukraine war, and the risk of climate events (Anonymous, 2021b). With an increase of 11 million tons in 2020/21, it reached the highest stock amount of 289 million tons in total. China, with a 17% share in the 289 million tons of stock, ranks first, followed by the EU and India, respectively. Türkiye ranked 10th in the world grain rankings with a share of 3% (Anonymous, 2021a). Wheat yield in the 2020/21 season; While there was a decrease in the European Union, Ukraine, the United States, India, and Argentina, an increase was observed in countries other than these countries compared with the previous seasons. In the 2020/21 season, wheat yield was determined to be 3.45 tons/ha, with a decrease of 1.7% (Anonymous, 2021a).

Wheat production, supply, use, trade, and ending stocks between 2017/2022 are shown in Table 1. As shown in Table 1, there has been an increase in grain production worldwide. Wheat production in states such as Russia, Canada, USA, and Brazil increased, and wheat production in states such as the EU, Ukraine, and Argentina decreased (Anonymous, 2020b).

When the world wheat cultivation areas are examined in Figure 1, five major producing countries come to the fore. These are the USA, China, EU, Russia, and India. These countries account for 44.9% of the world 's wheat cultivation area as of 2022. India ranks first, followed by Russia, the EU, China, and the United States.

Table 1. Wheat Grain Market (thousand tons)

Years	2017/18	2018/19	2019/20	2020/21	2021/22
Production	2.694.90	2.647.70	2.712.10	2.772	2.791.30
procurement	3.520.10	3.506.90	3.546.50	3.598.20	3.618.80
Using	2658.9	2.690.30	2.712.40	2.762.10	2.809.60
Trade	423.2	411.30	439.40	476.7	480.30
Expiring Stocks	859.2	834.50	826.20	827.5	822.10

Source: (FAO, 2021)

Table 2. World Wheat Data (thousand tons)

	2017/18	2017/18	2019/20	2020/21	2021/22	Annual rate of change (%)
Area (Thousand Ha)	218.475	215.439	216.654	221.848	223.790	0.9
Yield (Ton/Ha)	3.49	3.4	3.53	3.5	3.53	0.9
Production	762.557	731.552	764.156	776.097	788.978	1.7
Consumption	740.499	733.179	741.805	774.267	785.297	1.4
End of Year Stocks	287.816	284.084	299.439	294.667	294.962	0.1
Export	184.046	174.053	187.880	193.046	199.036	3.1
Import	185.427	176.158	194.876	199.648	202.422	1.4
Export Price ($/Ton)	211	241	219	239	284	19

Source: (Anonymous, 2021b)

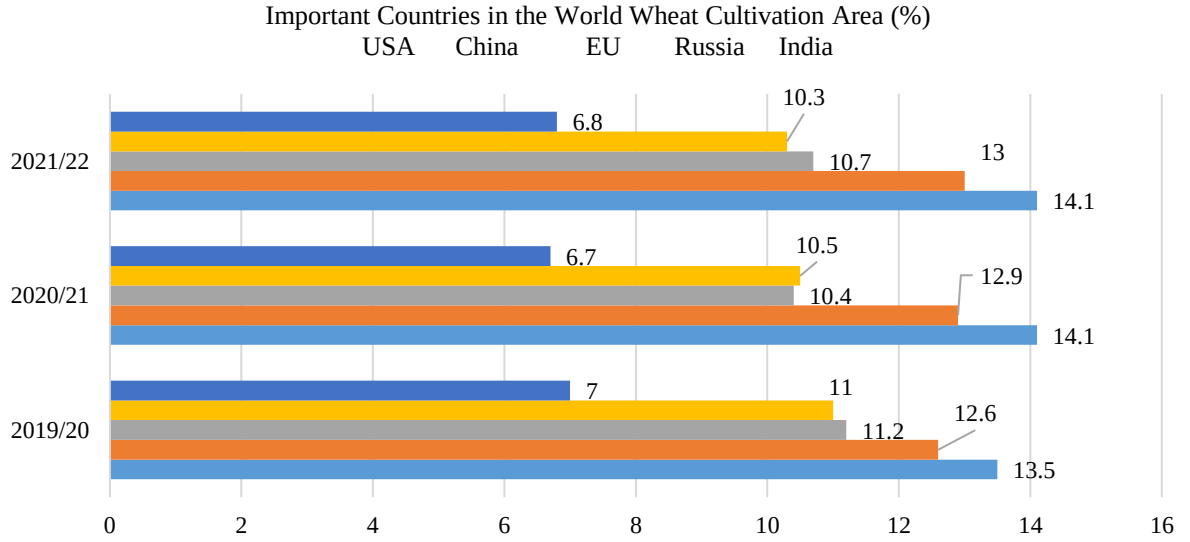

Figure 1. Share of Leading Countries in terms of Wheat Cultivation Areas in the World (Anonymous, 2021b)

It can be said that the leading countries in terms of cultivation area have a say in meeting world wheat demand and price formation. The countries listed in Figure 1 in terms of cultivation areas differ slightly in terms of production amount when their productivity levels are considered. While the largest producer in terms of production amount is China, it is followed by the EU, India, Russia, and the USA (Anonymous, 2021b). This situation may bring about some nativities in terms of the world 's general conjuncture and in today 's conditions where regional integration is negatively differentiated. In this case, Türkiye contributes to the processes with its regulatory and impressive mission due to its geography and strategic location.

Total grain production in 2021/22 is 2.8 billion tons. 28% of the production in question consists of wheat. 41% of grain exports consists of wheat. Russia, the EU, the USA, Ukraine, Canada, Australia, and Türkiye rank first in world wheat exports (Anonymous, 2021b). According to Ankara Commodity Exchange data, world wheat production increased in the 2020/21 season compared with the previous year and reached 763 million tons. Some balance has been achieved as wheat consumption increased in China and decreased in the United States. As the price increase in corn and barley increased the tendency toward wheat, the wheat trade between China and Pakistan in the 2020/21 season increased by 5 million tons compared to the previous year, reaching 189 million tons (Anonymous, 2021a).

Wheat is one of the most traded agricultural products because a significant portion of the world 's wheat volume is produced by certain countries, and wheat is the most consumed grain in the world (Gómez and Devadoss, 2004).

When recent years are examined in wheat imports of countries, it is seen that there has been an upward acceleration, and due to the Pandemic, production decreased in the 2020/21 season and the use of wheat as feed increased. This changed the demand for wheat, and imports increased compared with previous years (Anonymous, 2021a). Countries in international wheat trade are Egypt (13 million tons), Indonesia (10 million tons), China (10.5 million tons), Türkiye (9 million tons),

Algeria (6.5 million tons), Bangladesh (6.5 million tons). tons) and Brazil (6.6 million tons). Russia (about 40 million tons), EU (about 33 million tons), USA (about 25 million tons), Canada (about 23.5 million tons), Australia (about 21 million tons), Ukraine (about 20 million tons), and Argentina (approximately 13 million tons) stated that it is at the forefront in exports (Büyükkılıç, 2021).

When Türkiye 's wheat production in 2003/2018 is examined, it can be said that it is partially similar to world data (Table 6). Türkiye, which has an important place in the world wheat market, accounts for 3.24% of the world wheat economy (İstikbal, 2020). Looking at Table 4, while the cultivated areas in Türkiye have decreased over time since 2003, fluctuations can be seen in production.

According to the data of the Ministry of Agriculture and Forestry of the Republic of Türkiye, despite the negativities experienced in both logistics and supply chain due to the pandemic (COVID-19) seen in the world, the problems experienced did not turn into a food crisis. The increase in demand for wheat due to the pandemic has caused wheat prices to rise. To avoid any problems in food supply during the pandemic period in Türkiye, producers worked non-stop and produced 20.5 million tons, an increase of approximately 8% compared to 2019. In 2020, it amounted to 20.5 million tons, with an increase in approximately 8% compared with the previous year (Anonymous, 2021c).

According to TUIK data for the 2020/21 period, Türkiye 's wheat cultivation areas cover 3.2% of the world. Cereals constitute 70% of the total cultivated area in Türkiye. Wheat takes the first place among grains. Wheat production; In 2020/21, the cultivation area and yield increased by 7.9% to 20.5 million tons (Anonymous, 2021b).

Table 3 shows increases and decreases in the values in the planted area, production, usable production, consumption, and per capita consumption columns between 2009/19.

The Role of the TGB in the Grain Market of Türkiye

TGB was established in 1938 as an organization whose capital belongs to the state, is subject to the provisions of Decree Law No. 233 dated 08/06/1984, has legal personality, and has autonomy in its activities. TGB, which is affiliated with the Ministry of Agriculture and Forestry, is headquartered in Ankara and its capital is 2,550,000,000 TL (Anonymous, 2020a). TGB takes precautions by taking regulatory steps in the market to prevent grain prices from falling and producers from being victimized. It stocks products and sells existing stocks by ensuring balance in the market (Ekmen, 2019).

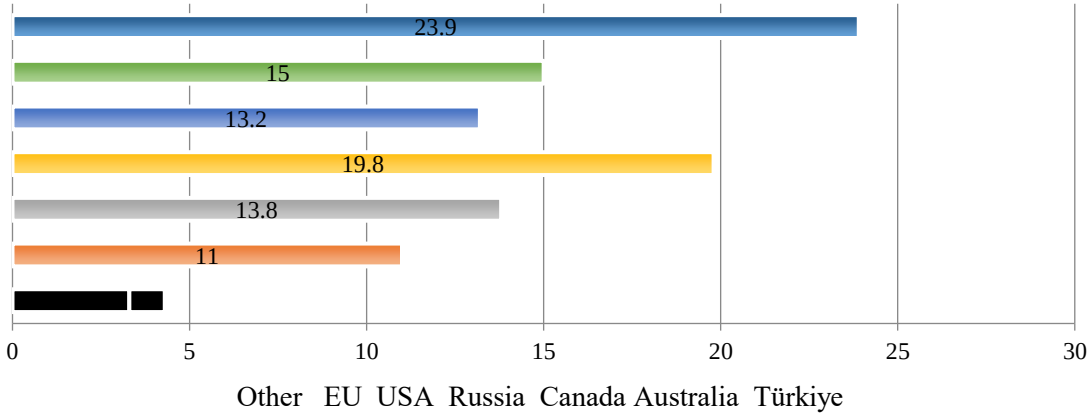

Figure 2. World Wheat Export percentage by Country (2020/21) (Anonymous, 2021b)

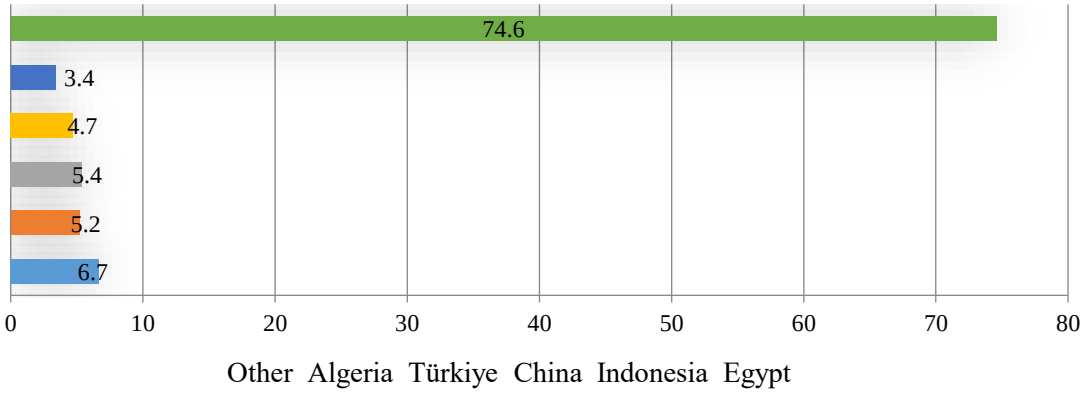

Figure 3. World Wheat Import percentage by Country (2020/21) (Anonymous, 2021b)

Table 3. Türkiye Wheat Cultivated Area, Production, and Consumption Data Between 2009 and2019 (TUIK, 2022)

Years	Cultivated Area (Hectare))	Production (Ton)	Usable Production	Total consumption (ton)	Per capita consumption (kg)	Usage (Ton)
2009	8.100.000	20.600.000	19.467.000	14.494.543	199,8	22.418.007
2010	8.103.400	19.674.000	18.591.930	15.766.287	213,9	22.766.035
2011	8.096.000	21.800.000	20.601.000	17.089.529	228,7	23.825.535
2012	7.529.639	20.100.000	18.994.500	17.042.330	225,4	23.024.199
2013	7.772.600	22.050.000	20.837.250	16.329.709	213	25.022.439
2014	7.919.209	19.000.000	17.955.000	15.604.364	200,8	23.735.716
2015	7.866.887	22.600.000	21.357.000	14.399.259	182,9	25.466.527
2016	7.671.945	20.600.000	19.467.000	14.490.08	181,5	24.053.405
2017	7.668.879	21.500.000	20.317.500	-	174,6	26.427.069
2018	7.299.271	20.000.000	18.900.000	-	179,4	25.367.562
2019	6.846.327	19.000.000	17.955.000	-	192,8	28.748.317

Table 4. Global Wheat Production and Türkiye (Million Tons / Hectare) (İstikbal, 2020)

Years	World Production	Total Cultivated Area	Production of Türkiye	Cultivated Area of Türkiye	Türkiye's share in Production	Türkiye's share in the Cultivated Area
2003	549.9	207.4	19	9.05	3.45	4.36
2005	627	221.6	21.5	9.22	3.42	4.16
2007	606.5	215.4	17.2	7.95	2.83	3.69
2009	683.6	225.1	20.6	8.02	3.01	3.56
2011	696.8	220.2	21.8	8.06	3.12	3.66
2013	710.3	218.8	22	7.75	3.09	3.54
2015	741.6	223.4	22.6	7.84	3.04	3.5
2016	748.3	219	20.6	7.6	2.75	3.47
2017	773.4	218.4	21.5	7.66	2.77	3.5
2018	734	214.2	20	7.28	2.72	3.39

While the TGB itself provides the purchase and sale of existing products, the organizations active with the TGB are cooperatives, and Agricultural Credit Cooperatives and Beet Growers Cooperatives (Pankobirlik) play an important role in the purchase, sale, and storage of grain (Ekmen, 2019). In addition to cooperatives, licensed warehouses have taken their place in the market since 2011 and purchase and store grain on behalf of the TGB. Licensed Warehouse was first established on 26/2/2010, in partnership with the Union of Chambers and Commodity Exchanges of Türkiye, with 50% shares in TGB-TOBB Agricultural Products Licensed Warehousing Industry and Trade Joint Stock Company. It is an organization with a capital of 100 million, headquartered in Ankara, in which the Product Specialization Stock Exchange also participates with 15% shares, by the Decision of the Council of Ministers dated 06/04/2017 and numbered 2017/9986 (Anonymous, 2020a).

78% of the purchases made in 2020 were made as ELÜS through TÜRB (Turkish Product Specialization Exchange). While TGB purchases within its own structure by appointment, all purchases in licensed warehouses are made without an appointment. A purchase price is given for each class of product. It purchased products up to 50% more than the production numbers of producers registered with FRS (Anonymous, 2020b).

Considered as a strategic product in terms of its production and usage areas, wheat's shockability allows taking action against possible risks. Although its production depends on natural conditions, it is directly affected by country policies because of its stockability. Country policies interact with producer behavior. Therefore, determining the behavior of producers at the micro level is considered important. In this research, the socioeconomic characteristics, preferences, and decisions in wheat production and marketing processes of wheat producers who deliver products to TGB through Licensed Warehouse A.Ş. in Mucur district of Kırşehir province, located in a strategically important region (Central Anatolia Region of Türkiye), are examined. 80% of the economy of Mucur District of Kırşehir province consists of agriculture. Wheat, barley, beet and sunflower etc. plantings are occurring. There are 803,627 ha of agricultural land as irrigated land (779,567 ha) and irrigated land (24,060 ha). These include 5,000 ha of vegetable-planted area, 3,000 ha of fruit-planted area, and 3,500 ha of vineyards. There are 500,000 ha of field and 110,000 ha of fallow land. The number of farmers registered with Farmer Recording System (FRS) is 3,500 (Anonymous, 2022a).

Material and Method

The main material of the study consists of surveys conducted with wheat producers who sell products to the TGB in the Mucur district of Kırşehir province.

In the research, it was determined that production was mainly performed with rainfed farming systems in terms of the geographical features and business typologies of the region. Producers mostly evaluate the products they produce in TGB and licensed warehouses. During the 2021 production period, the number of producers selling products to TGB through Licensed Warehouse Inc. (LİDAŞ) was 343 businesses (Kırşehir TGB records, 2021). Among the businesses in question, 114 producers (30% of the population) were purposely included in the sample. Among the surveys obtained from 114 producers, 14 surveys that were thought to be incomplete/erroneous were excluded, and the research was conducted with data collected from 100 producers. The Purposeful Sampling

Method is frequently used in agricultural economics studies (Çiçek and Erkan, 1996). The obtained data were interpreted by converting them into tables and graphs. Some variables deemed important were made into cross-tabulations, and a statistical chi-square analysis was performed. Cross tables are made according to the business size groups. Business size groups were planned to show the most appropriate distribution within the sample volume, and 0–65 da was expressed as the first group, 66–140 da as the second group, and 141-+ da was expressed as the third group.

Research Findings

Socio-Demographic Characteristics of Producers

When the sociodemographic characteristics of the producers were examined, it was determined that all of them were men, the majority of them were high school graduates in terms of education, there were more women in the household, and all of them had health insurance. It can be said that the grain production experience is approximately 21 years.

Factors affecting producers' wheat production decisions for the next year are given in Table 6. When the table is examined, it is determined that while the first group of enterprise size groups shapes the production decision according to the previous year 's wheat prices, the second and third enterprise size groups shape their production decisions according to the previous year's yield. According to the chi-square analysis, no statistically significant difference was observed in terms of factors affecting the determination of production decisions by business size groups. According to Aydın and Unaktan (2016), producers primarily base their decisions on their own experience and then take into account the experiences of experts. In the study by Doğan and Kan (2018), they investigated the effects of changes in temperature and precipitation in Türkiye between 1997 and 2016 on wheat yield. According to the results of their analysis, the yield was inversely proportional to temperature and had a positive relationship with precipitation. They stated that productivity can be increased by choosing the appropriate variety (Doğan and Kan, 2018).

Table 5. Socio-Demographic Characteristics of Producers

	n	%
Gender		
Male	100	100,0
Age(x̄)	47.58	
Education		
Literate	2	2.0
Primary School	10	10.0
Secondary School	19	19.0
High School	37	37.0
College	11	11.0
University	21	21.0
Total	100	100
Household Size (Excluding Business Owner)		
Male	85	36.15
Female	150	63.85
Health Assurance		
Agriculture Bağ-Kur	26	26.0
Pension Fund	14	14.0
SSK	60	60.0
Total	100	100
Annual Agricultural Income (x̄-₺)	176670.00	
Annual non-Agricultural Income (x̄-₺)	58486.84	
Agricultural Experience (x̄-year)	20.61	
Cereal Production Experience (x̄-year)	20.59	

Table 6. Factors Effective in Determining Wheat Production Decision One Year Ago

			WPP	AYP	NFM	BBA	AGNP
Business Size Groups	1. Group	n	16	14	7	16	5
		%	30.77	25.45	41.18	57.14	38,46
	2. Group	n	19	22	4	8	3
		%	36.54	40.00	23.53	28.57	23,08
	3. Group	n	17	19	6	4	5
		%	32.69	34.55	35.29	14.29	38,46
Total		n	52	55	17	28	13
		%	100	100	100	100	100
χ^2=37.918			P:0.217		P>0.05		

WPP: Wheat Price in the Previous Year; AYP: According to the Yield of the Previous Year; NFM: Newly-Formed Marketing Channels; BBA Not Being Affected by Anything; AGNP: According to the Guidance of Neighboring Producers; *Exceeds 100% because more than one option is marked.

Table 7. Characteristics Preferred by Producers in Wheat Varieties They Plant by Business Size Groups

	1. Group		2. Group		3. Grpup		Test and p Value
	n	%	n	%	n	%	
Certification of the Wheat Seed							χ^2=4.240 p=0.132
Yes	15	41.7	14	41.2	6	20.0	
No	21	58.3	20	58.8	24	80.0	
When choosing a variety, it is important that the variety I plant has a high yield.							χ^2=2.652 p=0.241
I agree	5	13.9	1	2.9	2	6.7	
Absolutely I agree	31	86.1	33	97.1	28	93.3	
The Quality of the Variety I Will Plant is Important in Variety Selection							χ^2=2.620 p=0.280
I agree	5	13.9	1	2.9	3	10.0	
Absolutely I agree	31	86.1	33	97.1	27	90.0	
The Market Selling Price of the Variety I Will Plant is Important in Variety Selection							χ^2=1.036 p=0.671
I agree	3	8.3	1	2.9	2	6.9	
Absolutely I agree	33	91.7	33	97.1	27	93.1	
When choosing a variety, it is important that the variety I plant is easy to market.							χ^2=2.563 p=0.836
I'm undecided	1	2.8	0	0.0	0	0.0	
I agree	2	5.6	3	8.8	1	3.3	
Absolutely I agree	33	91.7	31	91.2	29	96.7	
I Prefer to Plant the Variety I Am Used to							χ^2=8.475 p=0.306
I strongly disagree	0	0.0	0	0.0	1	3.3	
I do not agree	0	0.0	1	2.9	0	0.0	
I'm undecided	1	2.8	2	5.9	5	16.7	
I agree	8	22.2	5	14.7	5	16.7	
Absolutely I agree	27	75.0	26	76.5	19	63.3	
The variety I choose is resistant/tolerant of diseases and pests.							χ^2=1.888 p=1.000
I'm undecided	1	2.8	0	0.0	0	0.0	
I agree	5	13.9	4	11.8	4	13.3	
Absolutely I agree	30	83.3	30	88.2	26	86.7	
It is Important to Be More Resistant to Temperature							χ^2=0.555 p=1.000
I agree	2	5.6	1	2.9	1	3.3	
Absolutely I agree	34	94.4	33	97.1	29	96.7	
It is Important to Be Resistant to Low Water							χ^2=1.405 p=0.508
I agree	2	5.6	1	2.9	3	10.0	
Absolutely I agree	34	94.4	33	97.1	27	90.0	
It is Important to Use Less Fertilizer							χ^2=1.597 p=0.939
I'm undecided	1	2.8	0	0.0	1	3.3	
I agree	4	11.1	5	14.7	4	13.3	
Absolutely I agree	31	86.1	29	85.3	25	83.3	
Seed Price of the Variety I Will Prefer is Important for My Variety Preference							χ^2=4.229 p=0.375
I'm undecided	3	8.3	2	5.9	0	0.0	
I agree	7	19.4	5	14.7	9	30.0	
Absolutely I agree	26	72.2	27	79.4	21	70.0	
The Recommendation of the Place I Purchase the Seeds is Important in Variety Selection							χ^2=1.330 p=0.886
I'm undecided	1	2.8	2	5.9	2	6.7	
I agree	12	33.3	12	35.3	12	40.0	
Absolutely I agree	23	63.9	20	58.8	16	53.3	
I Prefer Varieties whose Seeds Are Easily Available							χ^2=11.847 p=0.009*
I'm undecided	2	5.6	3	8.8	0	0.0	
I agree	4	11.1	5	14.7	13	43.3	
Absolutely I agree	30	83.3	26	76.5	17	56.7	
It is Important to Have the Type Included in the TGB's Procurement Table							χ^2=5.308 p=0.491
I do not agree	0	0.0	1	2.9	0	0.0	
I'm undecided	2	5.6	3	8.8	6	20.0	
I agree	10	27.8	10	29.4	8	26.7	
Absolutely I agree	24	66.7	20	58.8	16	53.3	
Varieties for which TGBs Give High Prices According to Quality Analysis Are Important							χ^2=8.649 p=0.066
I'm undecided	1	2.8	3	8.8	8	26.7	
I agree	9	25.0	9	26.5	7	23.3	
Absolutely I agree	26	72.2	22	64.7	15	50.0	

83.3% of landowners with small wheat cultivation areas prefer varieties whose seeds are easily available, which is definitely effective in selection, 76.5% of those with medium land areas prefer varieties whose seeds are easily available, which is definitely influential in selection, 56% of large landowners prefer varieties whose seeds are easily available. It was observed that 7 of them preferred varieties whose seeds were easily available, which was definitely effective in the selection. According to the results of the chi-square analysis, there was a statistically significant relationship between the size of the wheat planted land and the preference for varieties whose seeds are easily available ($p < 0.05$).

38.9% of the owners of small lands with small wheat cultivation area consider it essential that the region of the wheat variety affects the price when selling wheat, 29.4% of the owners of medium land see it as crucial that the region of the wheat variety affects the price when selling wheat, and 29.4% of the owners of large land areas consider it essential that the region of the wheat variety affects the price when selling wheat. It was stated that 40.0% of the owners considered it unimportant that the region of the wheat variety affects the price when selling wheat. According to the results of the chi-square analysis, there was a statistically significant different between the size of the land planted on wheat and the state of thinking that the region of the wheat variety affects the price when selling wheat ($p < 0.05$).

44.4% of the owners of small lands with small wheat cultivation area consider it essential that the glassy grain ratio affects the price of wheat when selling, 61.8% of the owners of medium land see it as crucial that the glassy grain ratio affects the price when selling wheat, and 86% stated that 7.7% of them considered it essential that the glassy grain ratio affects the price of wheat. According to the results of the chi-square analysis, there was a statistically significant different between the size of the land planted on wheat and the belief that the glassy grain ratio affects the price of wheat when sold ($p < 0.05$).

The findings obtained according to some provisions regarding TGBs are as follows. 25.7% of the owners of small lands with wheat cultivation areas agreed with the statement that TGBs' purchasing and price policies affect their production decisions, 25.7% did not agree, and 32.4% of the owners of medium land areas agreed with the statement that TGBs' purchasing and pricing policies affect their production decisions. It was observed that 32.4% of the large landowners agreed with the statement that TGB's purchasing and price policies affect the production decision, and 30.0% strongly agreed with the statement that TGB 's purchasing and price policies affect the production decision. According to the results of the chi-square analysis, there was a statistically significant relationship between the size of the wheat planted land and the level of agreement with the statement that TGB's purchasing and price policies affect the production decisions ($p < 0.05$). No statistically significant different was found between the size of the wheat cultivated land and other characteristics and expressions related to the wheat market ($p > 0.05$).

Kan et al. (2017) found that their resistance to diseases, cold, and drought, as well as their suitability for family consumption (especially taste and taste), straw yield, and good straw quality are the most important reasons for choosing local wheat populations. Animal production is an important factor in the continuity of local wheat varieties in the region, and straw yield is more prominent than wheat yield in some regions. Tasci et al. (2022) in a study based on a 5-point Likert scale; They stated that the criteria that farmers think affect the sales of durum wheat are freedom from foreign matter, glassiness rate, hectoliter, semolina color, protein rate, moisture, and gluten quality, which have a significant impact on determining prices, and they found it to be crucial to have less foreign matter (Taşçı et al.). 2022).

In the study conducted by Özbek and Fidan (2013), in light of the analyzes made by the Konya Commodity Exchange and the surveys filled out by the producers, they determined that there were product losses and price reductions in wheat varieties due to diseases and pests (Özbek and Fidan, 2013). Tasci et al. (2020) conducted a study with producers producing durum wheat in the Yozgat region and listed the wheat variety as easily available, familiar, and recommended among the criteria preferred by the producers (Taşçı et al. 2020). James and Alston (2002) investigated policies in wheat fields in terms of yield and quality. According to the data they obtained, yield and quality indices were statistically significant in the distribution of wheat produced within quality classes (James and Alston, 2002).

The distribution of the effects of TGB's transition to the quality-based purchasing system in 2011 and the determination of grain prices on the producers of the producers participating in the research is given in Table 9. In the TGB's purchasing process using the quality-based protein analysis method, it was determined that 64 businesses in all groups did not make any changes in terms of business size. According to the results of the chi-square analysis, there was a statistical difference at the level of $p < 0.10$ in terms of business size groups compared with the quality-based purchasing system. According to the research of Tarhan and Dellal (2021), they stated that the TGB started to implement pricing practices based on protein-based quality criteria in wheat in 2011, causing the price of quality wheat to start trading at a higher value compared to other wheat (Tarhan and Dellal, 2021).

When the reasons of producers for wheat farming are examined, it can be said that there is no sales problem, the land is not suitable for growing other products, and it is an inherited habit. Regarding the wheat planted, it was observed that 24% preferred the generous type, 15% preferred the variety type, 14% preferred the Pehlivan wheat variety, and 11% preferred the Sönmez wheat variety. It has been determined that 60% of the producers participating in the research use their own seeds, while 46% supply them from the Agricultural Credit Cooperative, 22% from seed dealers, 59% use certified seeds and change their seeds every 3-4 years. It has been observed that when choosing wheat to plant, producers pay attention to high yield, quality, market sales price, ease of marketing, planting the variety they are used to, resistance to diseases and pests, water scarcity, low fertilizer need, and seed price The criteria that producers think affect the price when selling their wheat were examined and discussed in percentages. It has been observed that TGB is efficient and effective in determining purchase prices, that it has an impact on production decisions, that they are not satisfied with appointment working systems, and that they are satisfied with

payment planning. To be informed about innovations in agricultural production, producers mostly wanted to gather and provide training in provincial/district agricultural directorates and through the Internet and mobile phone applications. Producers sell their products to licensed warehouses, traders, and flour factories outside the TGB.

Table 8. Information on Wheat Marketing by Producers ' Business Size Groups

	1. Group		2. Group		3. Group		Test and p Value
	n	%	n	%	n	%	
Don't Have Trouble Marketing Wheat							χ^2=2.051 p=0.362
Yes	21	58.3	14	41.2	15	50.0	
No	15	41.7	20	58.8	15	50.0	
When Selling Wheat, DoNot Think that Wheat's Type Affects Its Price							χ^2=6.235 p=0.280
Insignificant	1	2.8	0	0.0	0	0.0	
Normal	0	0.0	1	2.9	1	3.3	
Important	11	30.6	6	17.6	11	36.7	
Critical	24	66.7	27	79.4	18	60.0	
When Selling Wheat, DoNot Think that the Region of the Wheat Variety Affects Its Price							χ^2=17.333 p=0.022*
Very unimportant	2	5.6	4	11.8	5	16.7	
Insignificant	6	16.7	5	14.7	12	40.0	
Normal	5	13.9	8	23.5	5	16.7	
Important	9	25.0	7	20.6	0	0.0	
Critical	14	38.9	10	29.4	8	26.7	
When Selling Wheat, DoNot Think that Protein Ratio Affects Its Price							χ^2=1.372 p=0.585
Important	4	11.1	3	8.8	1	3.3	
Critical	32	88.9	31	91.2	29	96.7	
When Selling Wheat, DoNot Think that Humidity Rate Affects Its Price							χ^2=4.079 p=0.775
Insignificant	1	2.8	0	0.0	0	0.0	
Normal	0	0.0	1	2.9	2	6.7	
Important	6	16.7	5	14.7	5	16.7	
Critical	29	80.6	28	82.4	23	76.7	
When Selling Wheat, DoNot Think that Hectolitre Affects Its Price							χ^2=0.345 p=0.917
Important	3	8.3	4	11.8	3	10.0	
Critical	33	91.7	30	88.2	27	90.0	
When Selling Wheat, DoNot Think that the Concentration of Foreign Matter Affects Its Price							χ^2=4.924 p=0.290
Normal	3	8.3	1	2.9	6	20.0	
Important	10	27.8	10	29.4	8	26.7	
Critical	23	63.9	23	67.6	16	53.3	
When Selling Wheat, DoNot Think that Semolina Color Affects Its Price							χ^2=9.351 p=0.107
Insignificant	1	2.8	0	0.0	0	0.0	
Normal	9	25.0	5	14.7	3	10.0	
Important	12	33.3	11	32.4	5	16.7	
Critical	14	38.9	18	52.9	22	73.3	
When Selling Wheat, DoNot Think that Vitreous Grain Ratio Affects Its Price							χ^2=15.640 p=0.006*
Insignificant	1	2.8	0	0.0	0	0.0	
Normal	8	22.2	4	11.8	0	0.0	
Important	11	30.6	9	26.5	4	13.3	
Critical	16	44.4	21	61.8	26	86.7	
When Selling Wheat, DoNot Think that Gluten Quality Affects Its Price							χ^2=8.823 p=0.166
Insignificant	2	5.6	0	0.0	4	13.3	
Normal	8	22.2	10	29.4	7	23.3	
Important	14	38.9	8	23.5	5	16.7	
Critical	12	33.3	16	47.1	14	46.7	
When Selling Wheat, DoNot Think that Sunk Sunk Destruction Affects Its Price							χ^2=4.480 p=0.724
Insignificant	1	2.8	0	0.0	0	0.0	
Normal	0	0.0	1	2.9	0	0.0	
Important	3	8.3	3	8.8	1	3.3	
Critical	32	88.9	30	88.2	29	96.7	
When Selling Wheat, Do Not Think that the Sale Date Affects Its Price							χ^2=6.647 p=0.559
Very unimportant	0	0.0	2	5.9	0	0.0	
Insignificant	2	5.6	1	2.9	0	0.0	
Normal	6	16.7	7	20.6	4	13.3	
Important	10	27.8	7	20.6	12	40.0	
Critical	18	50.0	17	50.0	14	46.7	

Table 9. The distribution of the Effect of TGB 's Transition to a Quality-Based Procurement System in 2011 and Determination of Grain Prices in this Way on Producers

			Changing the Varieties Used	Making Changes in the Cultivation Techniques	Not Making Any Changes	Not Being Aware of this Issue	Total
Business Size Groups	1. Group	n	2	2	23	9	36
		%	11.76	40.00	35.94	64.29	36.00
	2. Group	n	7	2	20	5	34
		%	41.18	40.00	31.25	35.71	34.00
	3. Group	n	8	1	21	0	30
		%	47.06	20.00	32.81	0.00	30.00
Total		n	17	5	64	14	100
		%	100	100	100	100	100.00
χ^2=14.741			P:0.064		P<0.10		

Conclusions and Recommendations

It has been observed that producers mainly plant wheat because of the influence of geography on their lands and the lack of irrigation facilities. Apart from wheat, the first product is the cultivation of barley, followed by cultivating sugar beet and chickpeas. It has been observed that 62% of the cultivation areas of these secondary products are property, 19% are rented, 71% are dry land, and 28% are irrigated land. It was determined that 63% of wheat lands were dry and 31% were irrigated. It can be stated that 37% of irrigated lands use sprinkler irrigation as an irrigation method. It was observed that in the year following the year in which they produced wheat, 54% of them planted barley, while 18% of them left fallow and preferred 2 or 3 years as an alternation year.

Based on the last five years, it has been determined that while a decrease in the cultivation areas of one-third of the producers has been observed, there has been no change in the cultivation areas of the majority. When we look at the producers' use of base, top, and leaf fertilizer, we see that they use bottom fertilizer (in October), top fertilizer (in March), and leaf fertilizer. It has been observed that while small land owners with small cultivation areas consider the region of the wheat variety as important when selling varieties whose seeds can be easily found, medium land owners consider the region of the wheat variety with easily available seeds, while large land owners prefer varieties with easily available seeds, which is definitely effective in the selection. They considered the region of the wheat variety unimportant and stated that the TGB's purchasing policies affected production decisions. One of the most important results of the research is that they are not satisfied with the TGB's purchasing practices for several reasons. The most important of these reasons has been determined that prices are reduced a lot in quality-based purchasing, and as a result, there is distrust in analysis applications. Quality-based purchasing system is the quality determination tests such as determining the protein ratio of the wheat brought by the producer using technological methods, determining the moisture level, and revealing its glassy properties. Price reductions resulting from this extremely rational practice are perceived by the producer as the TGB's price reduction policy. It is thought that explaining this situation to the producer with the right methods will have an impact on the trust in the institution. TGB is an interventionist organization that directs the grain market in Türkiye. The state is the biggest actor in market regulation and plays a role in creating producer and consumer balance by considering all dynamics. One of the most important results of this study is that the TGB and the Republic of Türkiye are more sensitive to local producers. Otherwise, it can be said that TGBs' trust in grain producers will decrease and their tendency to sell products to TGBs will decrease over time. It can be emphasized that the power of a TGB without a producer may become ineffective in the market over time.

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Effect of Time and Temperature Storage on the Quality of unpasteurized Prickly Pear Juice Enriched with Hydro-soluble *Opuntia ficus indica* seeds Extract

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ABSTRACT

This study aimed to evaluate the effect of incorporating hydrosoluble *Opuntia ficus indica* seeds extract in unpasteurized prickly pear juice and monitoring its stability. For this purpose, titratable acidity (TA), total soluble solids (TSS), browning index (BI), total phenolic compounds (TPC), total flavonoids (TF), antiradical activity (DPPH), ferric reducing antioxidant power (FRAP) and microbial analysis were monitored for both enriched and controlled juices during different time and temperature storage. Before storage, the enriched juice values were respectively 0.096±0.001%, 14.1±0.01%, 0.756±0.01, 133.3±3.4mgGAE/100ml, 5.58±0.07mgQE/100ml, 95.89±14.27mgGAE/100ml and 59.34±5.52mgGAE/100ml for TA, TSS, BI, TPC, TF, DPPH and FRAP; while 0.16±0%, 14.1 ±0.001%, 1.2±0.01, 88.39±4.2mgGAE/100ml, 3.98±1.003mgQE/100ml, 51.08±14.27 mgGAE/100ml and 50.33±5.16mgGAE/100ml for the control juice. The microbial analysis revealed the absence of microorganisms even the juices were unpasteurized. Moreover, the results revealed that the enrichment attenuated significantly the effect of storage; indeed, the use of the prickly pear seeds extract in combination with the juices can be a good alternative to enhance the shelf life of unpasteurized prickly pear juice, and improve their quality attributes as well as to minimize the unwanted changes in the nutritional and organoleptic properties.

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Introduction

The transformation processes, heat treatments, and storage have deleterious effects on the antioxidant potential of fruits and caused changes in the physicochemical properties and the levels of bioactive substances, which result in alterations in organoleptic, nutritional, and functional qualities (Touati et al., 2014; Touati et al., 2016; Zeeshan et al.,2019). Lately been gaining attention as a way to improve the nutritional value of food due to the increasing demand (Doménech-Asensi et al., 2013); hence, the food industry develops juice enriched with bio-substances to counterbalance the losses during processing and storage (Kaddumukasa et al., 2017).

The fruit of *Opuntia ficus indica* (OFI) is consumed as fresh fruit, juice, jam, and jelly; and it is a good source of bioactive compounds and may protect against human diseases. Moreover, cactus fruit juice positively affects the body's redox balance, and decreases oxidative lipid damage (Dehbi et al., 2014). The seeds of OFI play a key role in the valorization of the fruit because of their high antioxidants content. Their amount varied from 20 to 40% per dry weight of the whole fruit, depending on the cultivars (Habibi et al., 2002).

In Algeria, OFI is developing on the Mediterranean coast; but its culture is marginalized, its production is limited and its consumption remains seasonal. Despite its abundance, the prickly pear arouses little interest; it is not valued. The seeds are even less so since until now they are considered waste and yet their importance continues to grow in other countries such as Mexico, Argentina, and Spain (Habibi et al., 2005).

To our knowledge, several studies have been carried out on prickly pear pulp, seeds, cladodes, peels around the world (Al Juhaimi et al., 2020; Zeghad et al., 2019; Salehi et al., 2019; Smidaa et al., 2017; Chaalal et al., 2013; Habibi et al., 2005); however, there is no study on the monitoring stability of unpasteurized prickly pear juice enriched with its seeds extract.

Materials and Methods

Chemical reagents

DPPH reagent was purchased from Sigma Chemical (Sigma–Aldrich GmbH, Germany), and Folin–Ciocalteu phenol reagent from Biochem, Chemopharma (Montreal, Quebec). All chemicals and solvents used were of analytical grade.

Samples preparation

The fruits were harvested in Ain Hamra, department of Bordj Bou Arreridj (Latitude: 36°15′44’81″S, Longitude: 4°79′13’85″W) towards the end of August 2020. These fruits were stripped of their thorns, washed, peeled then kneaded with an electric mixer (SEB 500 Watt). After separating the seeds from the pulp, the latter was centrifuged (SIGMA 3-30KS) at 3000 rpm for 10 min. The filtrate recovered, constituting the juice, was split into two batches. The first batch was enriched with the hydro-soluble prickly pear seeds extracts (100 mg/l), while the second batch was considered as a control. Both lots were stored at 10,20 and 30°C. Samples for analysis were taken after 2, 4, 6, 8, 10 and 12 days.

Determination of physicochemical parameters

Titratable acidity

The determination of the titratable acidity consists in placing in a Beaker 10 ml of sample (juice) with a few drops of color indicator (0.1% phenolphthalein in pure ethanol). The reaction mixture was titrated with 0.1N NaOH solution until obtained a persistent pink color. The results were calculated according to the following equation:

$$TA\ (\%) = \frac{N_{NaOH}\ x\ V_{NaOH}\ x\ M_{citric\ acid}}{V_{sample}\ x\ 3\ x\ 10}$$

N_{NaOH}: molar concentration of NaOH, V_{NaOH}: volume of NaOH, $M_{citric\ acid}$: molar mass of citric acid, V_{sample}: volume of sample. The divide by 3 because citric acid is triacid (requires three molecules of NaOH to neutralize one molecule of citric acid); while the division by 10 is to express the results relative to 100 ml (Darias-Martín, et al., 2003).

Total soluble solids

The total soluble solids in a solution were measured with a refractometer. After placing a drop of juice on the surface of the glass plate, the value indicated represents the degree of brix expressed in percentage (%).

Browning index

The browning index was determined according to the method reported by Meydav et al. (1977). The samples were centrifuged (824×g, 18°C, 20 min); the recovered supernatants were diluted with ethanol (v/v) and then filtered through Whatman N° 2 paper. The absorbance was measured at 420 nm.

Determination of antioxidant substances

Total phenolic compounds

The total phenolic compounds of the juice samples was determined by the method using the Folin-Ciocalteu reagent (Adesegun et al., 2007). An aliquot of 100 µl of the extract was mixed with 800 µl of Folin-Ciocalteu (10%) and 400 µl of sodium carbonate (7%). After 30 min of incubation at room temperature, the absorbance was measured at 760 nm against the blank. The result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice by referring to the calibration curve.

Total flavonoids content

The total flavonoid content (TF) of the juice samples was determined by a colorimetric method (Ayoola et al., 2008). A volume of 2 ml juice was added to 2 ml of aluminum trichloride reagent $AlCl_3$ (2% in pure methanol). The absorbance was recorded at 420 nm after 10 min incubation at room temperature against the blank. The result was expressed in mg quercetin equivalent (QE) per 100 ml of juice by referring to the calibration curve.

Evaluation of antioxidant activity

DPPH radical scavenging capacity

The DPPH radical scavenging capacity was evaluated according to the method described by Brand-Williams et al. (1995). A volume of 200 µl of the sample was added to 1 ml of a methanolic solution of DPPH (60 µM). Absorbance was measured at 517 nm after 30 min incubation at room temperature and in the dark. The result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice by referring to a calibration curve.

Ferric reducing antioxidant power

The ferric-reducing antioxidant power was evaluated according to the method described by Oyaizu (1986). A volume of 2.5 ml of the juice sample was mixed with 2.5 ml of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of potassium ferricyanide (1%). After 20 min incubation at 50°C, 2.5 ml of trichloroacetic acid solution (10%) was added. A volume of 2.5 ml of the reaction mixture was diluted with distilled water (v/v) and then added with 500 µl of ferric chloride solution (0.1%). The absorbance was measured at 700 nm and the result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice referring to a calibration curve.

Microbiological analyzes

Preparation of dilutions

From the initial suspension (prickly pear juice), decimal dilutions were carried out under aseptic conditions.

Detection and enumeration of total coliforms

A volume of 1 ml of the sample was placed in empty Petri dishes prepared for this use and numbered. Then about 20 ml of medium (VRBG) was poured in. The tests were carried out in duplicate. A series of dishes were incubated at 37°C for 24 h. This will be used for the search for total coliforms.

Search and enumeration of yeasts and molds

Aseptically, 1 ml of juice was brought to a sterile and numbered petri dish. Then about 15 ml of medium (Sabauraud) was poured. Homogenization of the medium with the sample was made by 8-shaped movements. The tests were carried out in duplicate. The dishes were incubated at 25°C for 5 days.

Statistical analysis

The results (n = 3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($P<0.05$). All statistical analyzes were carried out using Infostat® software.

Results and Discussion

Evolution of physicochemical parameters

Titratable acidity (TA), total soluble solids (TSS), and browning index (BI) results of enriched and control juices during storage were shown in figure 1. Before storage, the enriched juice values were respectively 0.096±0.001%, 14.1±0.01%, and 0.756±0.01 for TA, TSS and BI; while 0.16±0%, 14.1±0.001% and 1.2±0.01 for the control juice. The present results were higher than that given by Dehbi et al. (2014), which reported TA values of 0,049%, for Marrocan prickly pear juice (Alkalaa cultivars with spiny, yellow peel, green-yellow pulp); while Medina et al. (2007) reported a rank of TA values between 0.055 and 0.078% for varieties harvested in the various regions of Bejaia. Stintzing et al. (2005) reported TSS values from 10 to 17% for prickly pear fruits of yellow-orange color. Chougui et al. (2013) reported a value of 15% for TSS of the prickly pear fruit. Concerning BI, the results fall in the range reported by Touati et al. (2016) inspite of differents kind of fruits proreported values of between 0.055 and 0.078% orange, pear, and grape nectar, respectively.Statistically, the enrichment before storage has a significant effect on TA and BI ($P<0.05$), contrary to TSS.

As can be seen from figure 1 a and b, TA values after 12 days of storage increased for both enriched and control juices to reach respectively. 1.12±0.003 and 2.08±0.002% at 10°C, 6.72±0.002 and 8.5±0.003% at 20°C, 6.69±0.04 and 8.3±0.02% at 30°C. This fact may be due to the fermentation (Jood et al., 2012). Ilkin et al. (2020) which worked on the stability of enriched orange juice with nettle (*Urtica dioica L)* during storage reported the absence of any statistical difference between the mean values of the samples.

Regarding TSS (figure 1 c and d), the values of enriched juice decreased significantly to achieve at the end of storage the values of 13.5±0.002, 13.0±0.002 and 12.3±0.1%, respectively at 10, 20 and 30°C; while for the control juice, the values were 13.6±0.001, 13.0±0.002 and 13.2±0.001%, respectively.

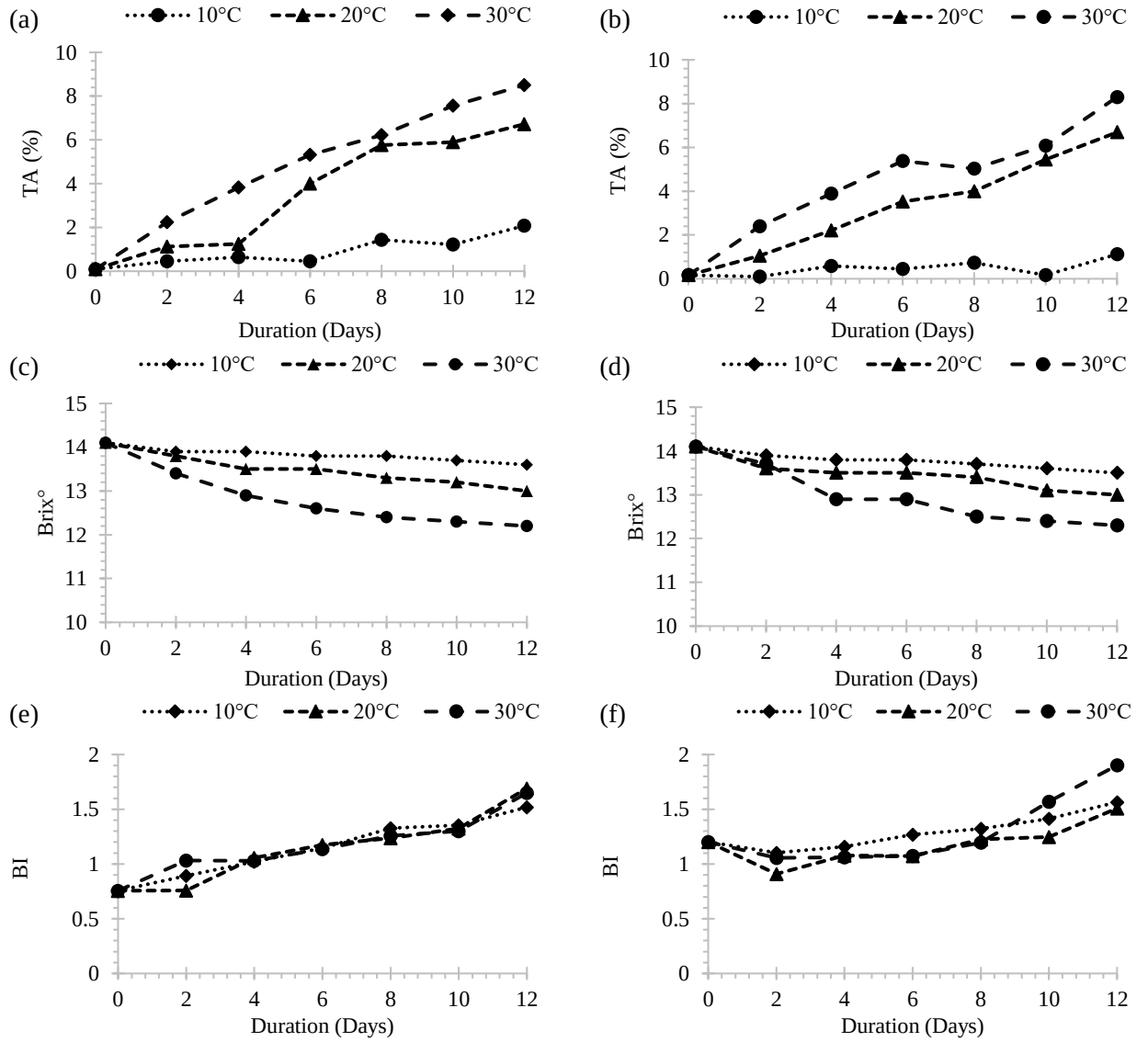

Figure 1. Evolution of physicochemical parameters of enriched and control juices respectively: Titratable acidity (TA), a and b; Total soluble solid (TSS), c and d; and Browning index (BI), e and f.

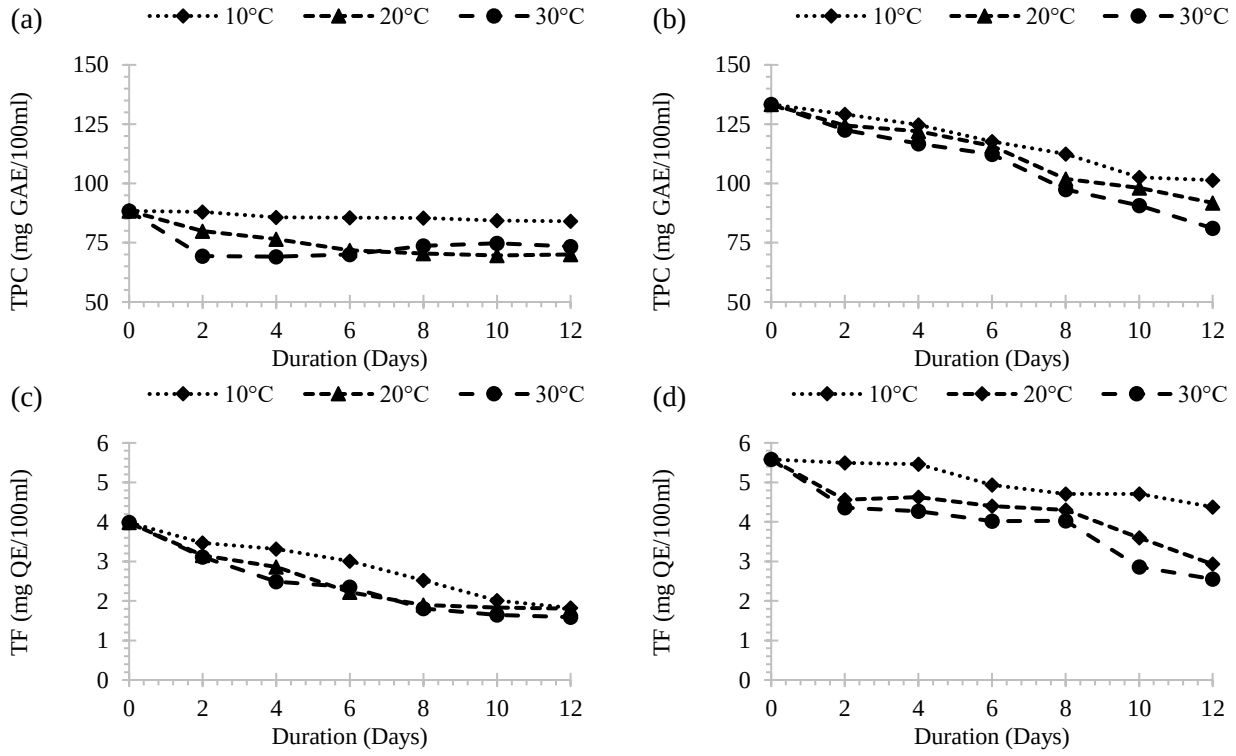

Figure 2. Evolution of antioxidants of enriched and control juices respectively: Total phenolic compounds (TPC), a and b; and Total flavonoids (TF).

The absorption of moisture during storage could be the reason for decreased in TSS values (Dangui, et al., 2014), or it could be due to the increase in the acidity (Sadras et al., 2013). The same results were found by Gao et al. (2018) who reported a decrease in TSS values of navel orange fruits during storage.

Concerning BI during storage at 10, 20 and 30°C (figure 1 e and f), the values increased respectively to achieve 1.519±0.06, 1.649±0.11 and 1.691±0.06 for enriched juice and 1.508±0.03, 1.565±0.01 and 1.902±1.002 for control juice. Touati et al. (2016) noted same trend in different fruits nectars. This augmentation may be due to the results of phenolic compound oxidation that occurred in the plant cell (Inchuen et al., 2010).

Evolution of the antioxidant substances

Total phenolic compounds

Total phenolic compounds (TPC), called secondary metabolites, are among the main antioxidants in plants, alongside vitamin C, vitamin E, and carotenoids. The results of TPC of enriched and control juices as well as their evolution during storage were shown in figure 2 a and b.

Prior storage, the content of TPC in enriched juice was 133.3±3.4mgGAE/100ml against 88.39±4.2mgGAE/100ml in control one. Therefore, the enrichment of juice induced an increase of 50.80% in the yield of TPC. Statistical analysis revealed that there was a significant difference between juices at $P<0.05$. The results obtained were higher than the values (31.0 - 51.1mgGAE/100g) reported by Palmeri et al. (2020). However, our results were lower than those reported by Socorro Santos Díaz et al. (2015) who declared that TPC values in the prickly pear juice were ranged from 630.9 to 880.6mgGAE/100ml. Dehbi et al. (2014) and Chavez-Santoscoy et al. (2009) reported values of 632.11±5.50µgGAE/g for the Marrocan *Opuncia ficus indica*. L juice and 226.3µgGAE/g for Mexican prickly pear juice, respectively.

As can be seen from figure 2a and b, the content of TPC in enriched juice stored at different temperatures exhibited the same decrease tendency, which was important for juices stored at 30°C followed by those stored at 20 and 10°C to reach respectively values of 101.4±0.005, 91.8±0.02 and 81.1±0.005mgGAE/100ml The content of TPC in the control juice stored at 10 and 20°C decreased significantly to reach values of 84.05±1.001 and 70.09±1.003mgGAE/100ml, respectively. In the juice stored at 30°C, a fluctuation was noted; a decrease during the first two days of storage, then stability until the sixth day, followed by an increase until the tenth day, then a decrease to reach the value of 73.42±0.003mgGAE/100ml. This may be due to leaching losses favored by the breakdown of cellular structures occurring as a result of exposure to high temperatures (Al Juhaimi et al., 2005). Several authors have found that TPC appears to exhibit stability during refrigerated storage while a decrease in ambient and high-temperature storage (Touati et al., 2016).

Total flavonoids

The total flavonoid (TF) content was determined using colorimetric method. The results of TF content in both enriched and control juices before and during storage were presented in figures 2c and d.

From figures 2c and d, TF content were 5.58±0.07 and 3.98±1.003mgQE/100ml for enriched and control juices, respectively. This indicates that the enrichment process led to a 40.20% increase in TF yield. Statistical analysis revealed a significant difference in the TF content between the analyzed juices at a significance level of $P<0.05$. The obtained results were higher than those reported by Zeghad et al. (2019) who stated the value of 1.95mgQE\g in prickly pear. On the other hand, our results were in concordance with values reported by Palmeri et al. (2020) who worked on prickly pear juice of different cultivars (4.7 and 5.7mgQE\g as TF in red and yellow cultivars).

The TF content in enriched juice stored at 10°C during the first four days did not exhibit a significant decrease (P<0.05); however, prolonged storage induced a significant decrease which led to reach the value of 4.38±0.008mgQE/100ml. Samples stored at 20 and 30°C showed a significant decrease during the first two days; while during extensive storage, the TF content showed slight stability until the eighth day followed by a significant decrease to reach values of 2.93±0.05 and 2.55±0.02mgQE/100ml for juice stored at 20 and 30°C, respectively. Concerning the control juice stored at different temperatures, the trend of reduction in TF content was greater for samples stored at 30°C followed by those stored at 20 and 10°C to reach the values of 1.82±0.02, 1.81±0,001 and 1.59±0.003mgQE/100ml, respectively. These results were consistent with the literature (Ogodo et al., 2016; Ali et al., 2013). The decline in TF content may be attributed to the breakdown of cell structure which occurred during the storage period (Ali et al., 2013). The decrease of TF content was lower in the enriched juice than the control one. This fact may be due to the addition of hydro-soluble prickly pear seeds extract

Evolution of total antioxidant capacity

Antioxidant contents have been reported to be the main responsible for foods total antioxidant capacity (TAC) (Touati et al., 2016). Therefore, TAC measurement could be a useful indicator of the quality deterioration of fruit juice during storage. For this purpose, DPPH and FRAP values of enriched and control juices were determined before and during 12 days of storage at 10, 20, and 30°C.

DPPH radical scavenging capacity

Results of the antiradical DPPH activity of enriched and control juices during storage was presented in figures 3 a and b.

Prior storage, the antiradical DPPH activity results were 95.89±14.27 and 51.08±14.27mgGAE/100ml for the enriched and the control juices, respectively. Statistical analysis revealed a significant difference between the analyzed juices (P<0.05). The enrichment process led to an increment of 46.73% in the antiradical DPPH activity. The total antioxidant capacity of prickly pear juices evaluated using the DPPH assay has been widely reported in the literature. Palmeri et al. (2020) reported DPPH results ranging from 37.6 to 49.4mgGAE/100ml for prickly pear juices. Smidaa et al. (2017) also noted that the antiradical DPPH activity increased with an increase in the concentration of Opuntia ficus indica.

As can be seen from figures 3a and b, the enriched juice exhibited a significant decrease in antiradical DPPH activity after 6, 8 and 4 days of storage at 10, 20 and 30°C, respectively. Extended storage up to the tenth day was characterized by stability, and then followed by a decrease to reach values of 46.28±1.006, 39.59±0.003 and 40.31±0.07mgGAE/100ml for juice stored at 10, 20 and 30°C, respectively. This might be explained by the decrease of antioxidants which where deteriorated during storage as corroborated by Tudora et al. (2015) who reported that under high temperatures storage some biochemical changes occurred in the fruit's structure. Regarding the control juice, the antiradical DPPH activity results were stable during the first two days of storage at the temperature of 10°C (P<0.05); however, the prolonged storage induced a significant decrease to reach the value of 25.06±0.006mgGAE/100ml. For juice stored at 20°C, the evolution of antiradical DPPH activity showed a decrease reaching a value of 20.79±1.04mgGAE/100ml at the end of storage. Concerning juice stored at 30°C, the values of antiradical DPPH activity showed a decrease during the first four days, and then followed by stability until the end of storage with a value of 23.87±0.06mgGAE/100ml. These findings indicate a degradation of antioxidants during storage, which could be attributed to the effects of temperature and other storage conditions.

Ferric reducing antioxidant power

Results of the ferric reducing antioxidant power (FRAP) of enriched and control juices before and during storage were presented in figures 3 c and d.

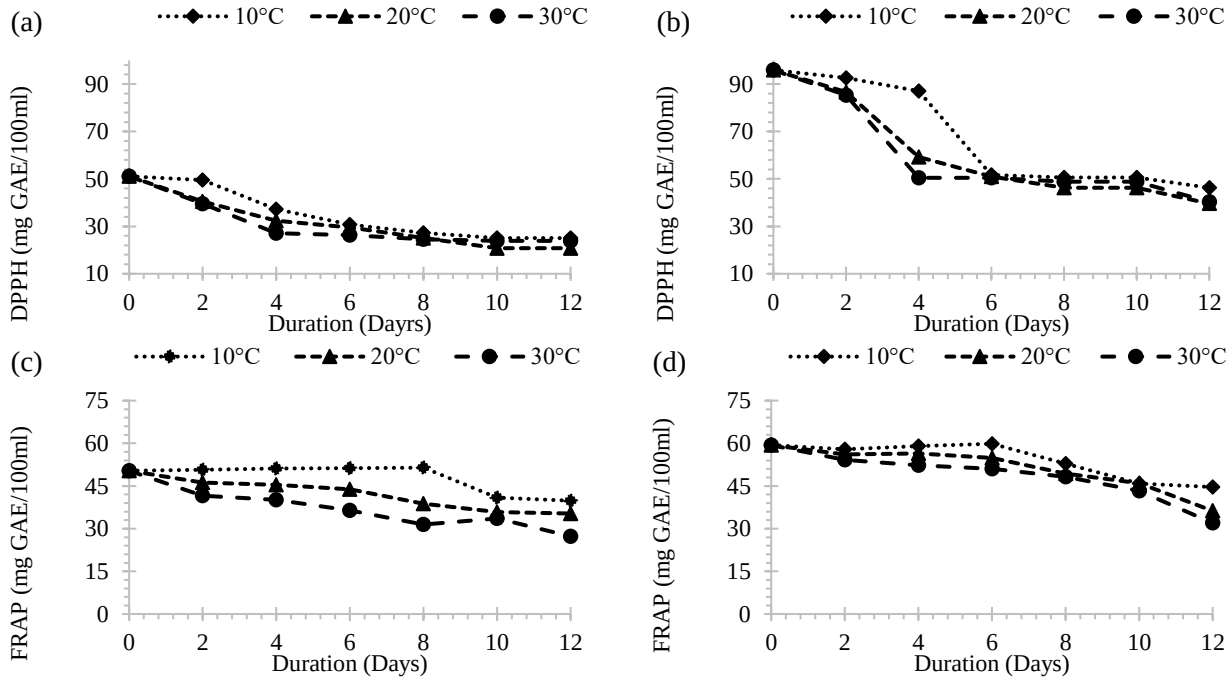

Figure 3: Evolution of antioxidant activities of enriched and control juices respectively: DPPH radical scavenging capacity (DPPH), a and b; and Ferric reducing antioxidant power (FRAP), c and d.

Table 1. Microbiological analysis results of enriched and control juices during storage

Germs	ST	Enriched juices				Control juices				Norms
		Prior storage	2 days	6 days	12 days	Prior storage	2 days	6 days	12 days	
Total coliform	10	-	-	-	-	-	-	-	-	< 10
	20	-	-	-	-	-	-	-	4.5×10^3	
	30	-	-	-	-	-	-	3×10^2	3.9×10^2	
Yeast and molds	10	-	-	-	-	-	-	-	-	10^4
	20	-	-	-	-	-	-	-	-	
	30	-	-	-	-	-	-	-	10.2×10^3	

ST : Storage temperature (°C)

Before storage, FRAP results were 59.34±5.52 and 50.33±5.16mgGAE/100ml for enriched and control juices, respectively. Therefore, the enrichment resulted in a 15.18% increase in FRAP. Statistical analysis showed a significant difference in the antioxidant activity between the enriched and control juices ($P<0.05$).

During storage, the FRAP values fluctuated for both analyzed juices; however, after storage, the temperature of 10°C induced less loss of reducing activity compared to juices stored at 20 and 30°C. The FRAP values at the end of storage under 10, 20 and 30°C were respectively 44.62±0.001, 36.26±0.001 and 31.97±0.003mgGAE/ 100ml for enriched juice, and 39.83±0.03, 35.31±0.005 and 27.32±0.007mgGAE/100ml for control one. The highest value was observed for the samples stored at 10°C

Microbiological analysis

Fresh juice made from fruits and vegetables is highly susceptible to contamination, leading to the deterioration of organoleptic and physicochemical parameters, (Sevindik et al., 2021). Several factors can affect microbial colonization of juices, including redox potential, pH, water activity, nutrients, temperature, antimicrobial agents, and relative humidity (Raybaudi et al., 2009; Pehlivan et al., 2018). The growth and survival of microorganisms in juices depend on their composition and the storage conditions. The results of the microbiological analysis of enriched and control juices, stored at three different temperatures (10, 20 and 30°C) for duration of 12 days, were presented in table 1.

Prior storage, as shown in table 1, results revealed the absence of contaminating germs (total coliforms, yeasts, and molds) in all samples, which perfectly meets the standards required by JORA (2017). These results were similar to those found by Garg et al. (2021) who worked on the Indian enriched gooseberry. Al Amin et al. (2018) reported the absence of total coliforms in orange and apple juice samples. Besides, Asghar et al. (2018) reported that unpasteurized juices such as apple, carrot, orange, and extracted sugar represent a high load of total coliforms.

During storage, the enriched juice showed the absence of total coliforms, which can be attributed to the antimicrobial activity of the aqueous extract of *Opuntia ficus indica* seeds extract. Previous studies have demonstrated the remarkable antibacterial effect of prickly pear seeds extract against various bacterial strains (Xiyu et al., 2020; Shimaa et al., 2022). Similar findings were declared by Al Amin et al. (2018), who reported the absence of total coliforms in commercial pineapple and lemon juice samples. In contrast, the control juices showed the presence of total coliforms, with a count of 3×10^2 CFU/ml after 6 days of storage at 30°C. This result was consistent with the findings of Lewis et al. (2006) and Rahman et al. (2011), which they reported the presence of total coliforms in juice samples. After prolonged storage, the total coliform count increased to 4.5×10^3 and 3.9×10^2CFU/ml for control juices stored at 20 and 30°C, respectively. The metabolic activities of microorganisms during storage can lead to the deterioration of juice samples and reduce their shelf life (Adal et al., 2022). Additionally, the low pH of the juice can promote the growth of acid-tolerant bacteria, further contributing to spoilage (Algari et al., 2016). According to JORA (1998) standards, the total coliform count in juice should be lower than 10CFU/ml.

Regarding yeasts and molds, the enriched juice remained free from their presence throughout the storage period at all temperatures (10, 20 and 30°C). This may be attributed to the high concentrations of secondary metabolites, such as flavonoids and polyphenols, present in the samples. These compounds can penetrate the cell membranes of fungal strains and interact with critical intracellular sites, leading to cell death (Cristani et al., 2007). In the control juice, yeasts and molds were absent in samples stored at 10 and 20°C; however, in samples stored for 12 days at 30°C, the number of yeasts and molds reached 10.27×10^3UFC/ml. The heat promotes the proliferation of yeasts and molds, and 30°C is considered an optimum temperature for their growth (Sevindik et al., 2021). These findings were align with the standards set by JORA (2017), which specify that, the yeast and mold count should be lower than 10^4UFC/ml.

Conclusion

In order to improve the stability of unpasteurized fruit juice, the latter has been enriched with the hydro-soluble extract *of Opuntia ficus indica* seeds. Regarding the physicochemical properties, no detectable difference between enriched and control samples throughout the storage period. In addition, the enriched samples exhibited the highest content of phenolic compounds and total flavonoids; likewise, the enriched juice has a higher antioxidant capacity. Furthermore, the seeds extract was effective in reducing the proliferation of microorganisms. The obtained results demonstrated the effectiveness of enrichment with the hydro-soluble prickly pear seeds extract to increase the nutritional value and improve the stability during storage. This research contributes to the development of innovative strategies in the juice industry by utilizing natural bio conservator and valorizing by-products, ultimately leading to the production of healthier and more sustainable juice products.

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Nutritional Composition and Apparent Metabolizable Energy Value of Black Soldier Fly Larvae (*Hermetia illucens* L.) Full-Fat Meal for Broiler Chickens[#]

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ABSTRACT

Black soldier fly larvae (BSFL; *Hermetia illucens* L.) is a proven high-cost protein source replacer and could be grown in a range of bio-degradable waste materials where hardly incorporated into broiler diets locally. The present study was aimed to assess the nutritional composition of BSFL, and apparent metabolizable energy (AME) value of BSFL meal provided to broiler chickens. BSFL full-fat meal produced from kitchen waste as a substrate were examined for their proximate composition, minerals and fatty acid profile. Eighty, 21-d old unsexed Cobb-500 broiler chickens (BW±SD: 665.8 ±14.3 g) were assigned randomly into 16 battery cages (04 replicates, five birds/replicate). A maize-soybean meal-based diet was used as the basal diet, which was partially substituted by pre-analyzed BSFL meal at the rates of 5%, 10%, and 15% to produce three test diets. Birds were fed in a completely randomized design for 7-d with a 4-d adaptation period. Excreta were collected for three days from day 25 to 28. The results envisaged that the crude protein (CP) and ether extract (EE) contents of the kitchen waste were 12.3%, and 10.5%, respectively. BSFL meal when analyzed had 34.4% CP and 47.3%, EE. The fatty acid (FA) profile of the kitchen waste was more or less similar to that of BSFL's meal. The estimated AME of the BSFL full-fat meal fed for broilers was estimated to be 15.7 MJ/kg. The BSFL full-fat meal can be utilized sustainably in feed formulation and has a high potential to replace costlier feed ingredients.

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Introduction

Producing food for increasing population is quite challenging in modern agriculture. According to the FAO (2009), current human population is expected to reach 9 billion in 2050. As a consequence, it has been estimated that the world's food production should increase by 70%. Concomitantly, protein supplementation through livestock products should be doubled than the current output (Schiavone et al., 2017). The broiler production is in front of fulfilling the protein gap efficiently and economically due to its faster growth rate and for excellent feed conversion ratio (Khusro et al., 2012). The broiler profit chain is marginalized, and the cost of production is mainly governed by the feed cost which accounts 70% of the total cost of production (Teguia and Beynen, 2005). The broiler performance which based on nutrient utilization is determined mainly by the respective diet's metabolizable energy to crude protein (CP) ratio (Zaman et al., 2020; Ravindran, 2013). Among the different feed ingredients that are used in boiler feed, the CP sources are the most cost demanded ingredients and are supplied by plant sources and animal by-products. However, some plant sources remain as staple foods in certain developing countries (Khusro et al., 2012).

The crop yield is known to be affected by population growth, climate change, and global warming scenarios (Dar and Laxmipathi Gowda, 2013). Additionally, the plant protein sources are also challenged with an imbalanced amino acid profile, lower CP content, and the presence of antinutritional factors (Bandara, 2018). Fish meal (FM) has been identified as one of the widely used animal-originated dietary CP sources, averaging 60 – 72 % of CP level (Cho and Kim, 2011). Rising global poultry feed requirement has been fortified to produce 7 to 7.75 million tonnes in 2022 (Lem et al., 2014). Fish meal is currently considered a scarce ingredient in many regions of the world; hence its price is inelastic. Numerous

environmental consequences have also created marine overexploitation (Bandara, 2018). Therefore, the poultry feed industry is always encouraged to explore alternative CP sources for sustainability.

The potential of insects in the livestock feed sector has been documented in past research, evidencing a positive influence on broiler production (Józefiak et al., 2016; Khan et al., 2018; Onsongo et al., 2018). This application establishes a sustainable approach, ensuring positive environmental impacts; less energy, land area requirement, and less environmental footprint for production (Makkar and Ankers, 2014). The black soldier fly larvae (BSFL, *Hermetia illucens* L.) is a promising insect for industrial poultry feed production. Its larvae thrive on a diverse range of waste materials that are unsuitable for human consumption. The larvae utilize very dense populations of organic wastes as varied as manures (Sheppard et al., 1994; Liu et al., 2008), rice straw (Zheng et al., 2012), food waste (Schiavone et al., 2017), distillers' grains (Webster et al., 2016), fecal sludge (Lalander et al., 2013; Banks et al., 2014), and animal offal and kitchen waste (Schiavone et al., 2017). Other insect larvae such as crickets and mealworms have lower feed conversion percentages. Black soldier fly larvae can convert these substrates into high-quality protein with a dry matter content ranging from 38 to 46%. Black soldier fly larvae's amino acid (AA) profile is high in methionine and lysine (9.05 and 22.3 g/kg DM, respectively) (Oonincx et al., 2015). It has been compared to or outperformed soybean meal which is the most popular and commonly used plant originated protein ingredient for poultry (Veldkamp et al., 2012).

Organic waste is common in low-to-middle-income countries, whereas paper, metals, and glasses are common in high-income countries. Recycling, incineration, waste-to-energy conversion, composting, and landfilling are the most well-known waste disposal processes available worldwide (Nanda and Berruti, 2021). Global municipal solid waste generation is predicted to be 2 billion tonnes, with an average of 0.74 kg/cap/day of garbage generation, with 33% of the waste remaining uncollected by municipalities (Kaza et al., 2018). Sri Lanka creates 7410 tonnes of municipal waste every day, where it has a higher fraction of organic materials with high moisture content. As a result, municipal solid waste is a recyclable and a cost-effective resource with great potential for addressing complex environmental concerns (Basnayake, 2019). The BSFL is a non-pest with a more extended larval period that allows for higher bio-accumulation through a wide variety of organic degradation. Because of the low level of technological readiness and economic feasibility of manufacturing, the BSFL's organic bio-conversion can be positively incorporated into the livestock feed industry (Čičková et al., 2015).

Scott and Boldaji (1997) have experienced in utilizing acid insoluble ash (AIA) as an inert marker in broiler digestibility experiments, where they found it as more suitable than synthetic chromium oxide. Nonetheless, data on BSFL digestibility and apparent metabolizable energy (AME) of BSFL for broilers is highly limited at the moment, limiting the formulation of sufficient BSFL-based broiler diets. Therefore, this study aimed to assess the nutritional composition and AME of BSFL full fat meal provided to broiler chickens.

Materials and Methods

The study was conducted at the farm premises of the Faculty of Agricultural Sciences, the Sabaragamuwa University of Sri Lanka according to the guidelines of the current ethical review committee (Reference no. ERC/A/01/2022/01 dated 15/09/2022) of the Sabaragamuwa University of Sri Lanka.

Preparation of the BSFL meal

Kitchen waste containing food and refuses were used as the common waste substrate to grow the BSFL (Ellewidana et al., 2020). The pre-pupae stage of BSFL were harvested, cleaned, and were stored under -20℃ until further processing. At the collection point, the larvae weighed around $0.21 g \pm 0.02$ and $22.1 mm \pm 4.7$ in average length. The frozen larvae were thawed to room temperature and forced air dried for 20 hrs under 60℃ in a convection oven (Model: BOC-V640F, Biobase Biodustry (Shandong) Co; Ltd, China) (Schiavone et al., 2017). The dried larvae were ground into a fine powder using a kitchen grinder and were stored under 4 ℃ until mixed into diets.

Experimental Diets

A maize-soybean meal-based diet was used as the basal diet (NRC, 1994). The basal diet was partially replaced by pre-analyzed BSFL meal at the rates of 5%, 10% and 15% to produce three test diets.

Table 1. Ingredients and the calculated composition of the basal diet (g/kg as fed)

Ingredient	g/kg
Maize	485.8
Soybean Meal (44% CP)	224.7
Rice Polish	150.0
Fish Meal-Anchovy	62.6
Coconut Oil	50.0
Limestone Powder	13.0
Di-Calcium Phosphate	4.5
Common Salt	2.5
Vitamin Premix[1]	2.5
Mineral Premix[1]	2.5
L-Lysine HCL	0.84
Dl-Methionine	0.56
Coccidiostat	0.50
Calculated composition	
Metabolizable Energy (MJ/kg)	13.4
Dry Matter, %	90.1
Crude Protein, %	20.0
Ether Extract, %	8.99
Crude Fiber, %	3.32
DL-Methionine, %	0.44
L-Lysine HCl	1.20
Calcium, %	0.91
Total Phosphorus, %	0.72
Non Phytate Phosphorus, %	0.36

[1]Vitamin and mineral premixes (IU/mg per kilogram): Vitamin A 15,000,000; Vitamin D 3,800,000; Vitamin E 30,000; Vitamin K 2,500; Vitamin A 15,000,000; Vitamin B 1 2,500; Vitamin B 2 6,000; Calcium Pantothenate 12,000; Vitamin B 6 5,000; Vitamin B 12 24; Niacin 40,000; Folic acid 1,200; Biotin 180; Choline Chloride 2,000; Iron 40,000; Copper 10,000; Zinc 60,000; Manganese 80,000; Iodine 1,000; Cobalt 200; Selenium 150.

Chemical Analysis

The kitchen waste substrate samples where the larvae were reared were analyzed for dry matter (DM), Ash, CP, EE, gross energy (GE), calcium (Ca), total phosphorus (TP) contents and for its fatty acid (FA) profile. Black soldier fly larvae meal was analyzed in triplicates for its proximate composition, GE, Ca, TP, and FA profile (AOAC, 2005). For the determination of AME, the GE (AOAC, 2005) and AIA (Vogtmann et al., 1975) of test diets and excreta samples were analyzed in triplicates.

Estimation of AME of BSFL full-fat meal for broiler chickens

A total of hundred and fifty (150), vaccinated, unsexed, day-old broiler chicks (Cobb 500) were purchased from a commercial hatchery in Sri Lanka. They were raised in floor pens and were fed a commercial diet (12.5 MJ/kg and 21% CP) till day-21. On day 21, birds were individually weighed (665.8 ±14.3 g), and eighty (80) birds having uniform weights were randomly distributed into 16 battery cages (60 cm × 60 cm x 60 cm) (four replicates, five birds per cage). From day 21 to day 28, birds were fed with four test diets. The ingredient composition of the basal diet and its calculated composition are indicated in Table 1. The feed intakes throughout the experimental period were recorded. The collection trays were introduced on day 25 and lasted after three consecutive days. Daily excreta collected from each cage after removing feathers and feed residues were weighed, labeled, and were stored at -20^{0}C. Daily collections were pooled within a cage and were forced air-dried (Model: BOC-V640F, Biobase Biodustry (Shandong) Co; Ltd, China) until constant weights were obtained and were ground into a fine powder.

Calculations

The AME (MJ/kg diet) value of each diet in each inclusion level was calculated using Equation 1 as described by Scott and Boldaji (1997).

$$\text{AME} = \text{GE diet} - [\text{GE excreta} \frac{\text{Marker diet}}{\text{Marker excreta}}] \quad (1)$$

GE = gross energy, MJ/ kg (diet, excreta)
Marker = concentration of AIA

AME value of BSFL meal at each inclusion level was calculated using Equation 2 (Wu et al., 2020).

$$AME_d = AME_{bd} + (AME_{bd} - AME_{BSFL}) \times P_{BSFL} \quad (2)$$

Where; AME_d= AME diet, AME_{bd} = AME basal diet, AME_{BSFL} = AME Black Soldier Fly Larvae, P_{BSFL}= BSFL Proportion %.

The AME of BSFL meal was finally calculated by obtaining the mean value from obtained three AME values of BSFL meal.

Results and Discussion

Nutrient composition of kitchen waste substrate and full fat BSFL meal

The nutrient composition of rearing substrate and harvested BSFL meal are presented in Table 2. Nutrient composition of BSFL varies with the quality of the growing substrate.

Analytical data on nutritional composition of BSFL rearing substrates are highly limited. Kierończyk et al. (2020) and Shumo et al. (2019) documented the nutritional composition of BSFL reared on a similar growing substrate but resulted in different nutrient compositions (Table 3).

The effect of different kitchen waste substrates on the nutrient composition of BSFL is summarized in Table 4. The nutrient composition of BSFL was in agreement with the values obtained by previously researchers. The highest EE level was reported from the current experiment. The CP content reported in the present study can suppress some commonly used feed ingredient such as sunflower meal (Willis, 2003). The body composition of BSFL is known to influence by the quality and the quantity of food consumed (Nguyen *et al.,* 2015). Therefore, it is suggested that the composition of the rearing medium should be well balanced (Kierończyk et al., 2020). Moreover, the nutrient percentage of BSFL is also influenced by the larval growth stage. Considering the CP percentage of BSFL, Rachmawati et al. (2010) reported a decline in their growth stage (5 days old: 61%; 15 days old: 44% and 20 days old: 42% larvae).

Variations in nutritional composition of BSFL in diverse waste substrates other than kitchen waste have been well documented. The larvae fed on cattle manure (Newton et al.,1977; Li et al.,2011), swine manure (Newton et al., 2005; St-Hilaire et al., 2007; Li et al., 2011; Manzano-Agugliaro et al.,2012), chicken manure (Sheppard et al., 1994; Li et al., 2011), chicken feed (Bosch et al., 2014; Nguyen et al., 2015; Oonincx et al., 2015), palm kernel meal (Rachmawati et al., 2010), liver (Nguyen et al., 2015), fish (Nguyen et al., 2015) and Barry (2004) had varying CP (40 – 62.7%) and EE (6 – 49%) percentages.

Table 2. Analyzed nutrient composition of the kitchen waste substrate and the black soldier fly larvae meal on dry matter basis

Nutrient	Kitchen Waste Substrate (% DM)	BSFL Meal (% DM)
Crude Protein	12.3	34.4
Ether Extract	10.5	47.3
Crude fiber	-	5.97
Dry matter	92.0	92.7
Calcium	0.42	0.46
Total Phosphorus	0.50	1.20
Crude Ash	12.9	9.57
Gross Energy (MJ/kg)	17.0	17.8

Table 3. Comparison of some published data on nutrient composition of different substrates used for Black soldier fly larvae on dry matter basis

Reference	CP	EE	CF	DM	C	TP	CA	Substrate
The present experiment	12.3	10.5	-	92.0	0.42	0.50	12.9	KW
Kierończyk et al. (2020)	13.0	1.30	8.73	-	-	-	5.75	KW
Shumo et al. (2019)	20.0±0.5	7.2±0.3	-	92.7±0.1	-	-	7.20±0.3	KW
Spranghers et al. (2016)	8.60	2.10	33.6	-	0.683	0.293	10.8	Vegetable waste
Spranghers et al. (2016)	15.7	13.9	4.1	-	0.141	0.237	4.50	Restaurant waste

CP: Crude protein (%); EE: Ether extract (%); CF: Crude Fiber (%); DM: Dry Matter (%); C: Calcium (%); TP: Total Phosphorus (%); CA: Crude Ash (%); KW: Kitchen waste (Mixture of Wheat bran, Carrots, Cabbage, and Potatoes)

Table 4. Comparison of some published data on nutrient composition of Black soldier fly larvae utilized different substrates on dry matter basis

Reference	CP	EE	CF	DM	C	TP	A	Substrate
The present experiment	34.4	47.3	5.97	92.7-	0.46	1.20	9.57	KW
Kierończyk et al. (2020)	45.4	14.0	9.83	-	-	-	8.16	KW
Rawski et al. (2020)	35.0	29.8	7.90	-	-	-	5.30	FVF
Jansen (2018)	36.1	42.9	8.10	93.3	4.29	0.72	11.9	KW
Shumo et al. (2019)	33.0±1.0	34.3±0.4	-	87.7±1.0	1.93±0.42	2.0±0.58	9.6±1.6	KW
Spranghers et al. (2016)	39.9	37.1	-	-	2.87	0.404	9.6	Vegetable waste
Spranghers et al. (2016)	43.1	38.6	-	-	0.123	0.408	2.7	Restaurant waste

CP: Crude protein (%); EE: Ether extract (%); CF: Crude Fiber (%); DM: Dry Matter (%); C: Calcium (%); TP: Total Phosphorus (%); A: Ash (%); KW: Kitchen waste (Mixture of Wheat bran, Carrots, Cabbage, and Potatoes); FVF: Fresh vegetable and fruit mix

The crude fiber (CF) content of BSFL reported in the present study is comparatively lower (5.97%) than the values reported by Jansen (2018) (8.10%), and Newton et al. (2005) (7%) who used pig manure as the rearing substrate. Diets high in fiber are favorable in feed application as they influence the mucosal lining of the intestine (Montagne et al., 2003). Moreover, fiber has been found to favor the ensuing effects on the intestinal mucus barrier of chickens (Sumbule et al., 2021). According to Mwaniki et al. (2018), high feed intake by birds fed insect-based diets may be related to increased fiber content at different BSFL inclusion levels. Fibers aid in increased ceca fermentation in birds, resulting more excellent nutrient absorption and development (Bovera et al., 2016). Also, CF content could have an influence on protein digestibility (El-Wahab et al., 2021). Therefore, growing economic insect-based feeds might be more beneficial if fiber percentages were mixed in various feeds. It is practiced that feeding fiber to rations at low levels has a positive effect, but levels more than 3% in a ration have been proven to impact voluntary feed intake significantly and nutrient digestibility, resulting poor bird performance (Tejeda and Kim, 2021).

Additional to major nutrients, Ca and TP are known to ensure physiological functions of birds; egg shell formation, and maintenance of bones (Jansen, 2018). Calcium and TP deficiency can cause bone loss, stunted growth, and poor posture (Shumo et al., 2019). The present study reported comparatively lower levels of Ca (0.42%) and TP (1.2%) than the values reported by other researchers (Table 4) (Newton et al., 2005; Spranghers et al., 2016). Influence of the rearing substrate on the mineral composition of BSFL is well documented. Moreover, the outer layer of the skin of BSFL secretes calcium carbonate ($CaCO_3$), thus affecting the mineral concentration (Shumo et al., 2019). The ash content of BSFL revealed in the present study (9.57%) is in a similar range to that of previous studies (Table 4). However, it has been revealed that the crude ash content may influence the fat digestibility variably (El-Wahab et al., 2021).

The analyzed lipid profile of the kitchen waste or the rearing substrate and the BSFL full fat meal are presented in Table 5. The results indicated that the FA profile of the substrate is more or less similar to that of full fat BSFL meal. It is suggested that the FA profile of BSFL is reflected by the FA profile of the substrate (Makkar and Ankers, 2014; Spranghers et al., 2016). According to the results, lauric acid, palmitic acid, myristic acid, oleic acid, linoleic acid, and stearic acid were dominant in BSFL meal. The FA concentrations are in line with the previous studies where BSFL were grown in similar and or different rearing substrates (Sealey et al., 2011; Spranghers et al., 2016; Barragan-Fonseca, 2018). Saturated fatty acids (SFA) are composed of palmitic and myristic acids, which enhance the low-density lipoproteins (LDL) by suppressing the LDL receptors' expression (Sacks and Willett, 1991). Unsaturated fatty acids are more favorable in poultry diets and are abundant than SFAs in BSFL meals. More importantly, a higher lauric acid content (53.04%) of BSFL meal reported in the present experiment is more or less similar to those reported by Spranghers et al. (2016) (60.8% in vegetable waste; 57.5% in restaurant waste) and St-Hilaire et al. (2007) (49.37% in swine manure). Lauric acid is a natural antimicrobial substance, which can suppress lipid-enveloped viruses, many pathogenic bacteria, and protozoa (Spranghers et al., 2016). Therefore, BSFL meal-enriched diets are hygienic and are possibly abolish the usage of synthetic antibiotics in poultry diet formulae.

Apparent metabolizable energy (AME) of BSFL meal

To the best of author's knowledge, the research conducted to estimate AME of BSFL are highly limited. The AME of BSFL at 5%, 10% and 15% inclusion levels in the present study were estimated to be -0.49, 25.8 and 15.7 MJ/kg, respectively (Table 6). However, negative AME values of BSFL meal were not been reported previously. Based on the past avian feeding experiments, only very few studies have published negative AME values for feed ingredients (Table 7). According to Sibbald (1982), the inclusion level of the test material is crucial for estimating the AME value of a test ingredient.

Table 5. Analyzed lipid profile of kitchen waste substrate and Black soldier fly larvae meal (g/ 100g of fat)

Fatty acid	Kitchen Waste Substrate	BSFL Full Fat Meal
Caproic acid (C 6:0)	0.07	ND
Caprylic acid (C 8:0)	1.90	ND
Capric acid (C 10:0)	2.80	0.94
Lauric acid (C 12:0)	33.4	53.0
Tridecanoic acid (C 13.0)	ND	0.02
Myristic acid (C 14:0)	14.7	12.6
Palmitic acid (C16:0)	16.4	13.1
Palmitoleic acid (C 16:1 c)	0.51	2.29
Heptadecanoic acid (C17:0)	0.07	0.05
Cis-10- Heptadecanoic acid (C17: 1c)	0.05	0.03
Stearic acid (C 18:0)	3.48	1.76
Elaidic acid (C18: 1 9t)	0.10	ND
Oleic acid (C18 :11 9 C)	15.8-	10.9
C 18:2 (9C,12t)	0.05	ND
C 18:2 (9t, 12c)	0.04	ND
Linoleic acid (C 18:2 n6c)	8.49	4.42
C 18:3 (9t,12t,15c + 9t, 12c, 15t)	0.22	0.13
Cis-11-Eicosenoic acid (C 20:1)	1.06	ND
Linoleic acid (c 18: 3n3)	0.23	0.52
Heneicosanoic acid (C21:0)	ND	ND
Cis-11, 14-eicosadienoic acid (C 20:2)	ND	ND
Behenic acid (C22:0)	ND	ND
Cis-8,11,14- Eicosatrieonic acid (C20:3n6)	0.12	0.06
Erucic acid (C22:1n9)	0.04	ND
Cis-11,14,17- Eicosatrieonic acid (C20:3n3)	0.46	0.03
Lignoceric acid (C 21:0)	ND	0.02

BSFL, Black soldier fly larvae; ND, Not detected.

Table 6. Apparent metabolizable energy of full fat Black soldier fly larvae meal at different substitution levels

Criteria	Basal diet	Diet $_{BSFL\ 5\%}$	Diet $_{BSFL\ 10\%}$	Diet $_{BSFL\ 15\%}$
Average AME (MJ/kg)[1]	8.95	8.62	10.32	9.83
SEM $_{AME\ diet}$	0.30	0.99	1.13	0.99
AME_{BSFL} (MJ/kg)	-	-0.49	25.82	15.66
Dry Matter %	82.92	84.35	83.96	85.37

[1]Each value represents the mean of four replicates. AME, Apparent metabolizable energy.

Table 7. Negative apparent metabolizable energy values for feed ingredients reported in the past research studies

Reference	Feed ingredient	Inclusion level (%)	[1]AME value	Method	Bird type
Potter et al. (1960)	Alpha cellulose	0, 20, 33.3	(-0.189) ± 0.062 cal/g	Multiple regression method	White Plymouth Rock chicken
Sugden (1973)	Aquatic liverwort (Ricciocarpus natans)	30.7	(-0.06) ±0.05 kcal /g	Marker method	Ducks
	Dock fruits (Rumex maritimus)	39.8	(-0.37) ±0.07 kcal /g	Marker method	Ducks
	Pondweed foliage (Potamogeton Richardsonii)	40.2	(-0.45) ±O.05 kcal /g	Marker method	Ducks
Petersen et al. (1976)	Barley hulls	25	(-0.29±0.18) kcal /g	Marker method	4-week-old male broiler chicks
	Barley hulls	25	(-0.22±0.13) kcal /g	Marker method	4-week-old female broiler chicks
Ortiz et al. (2001)	Linseed	16	–2·96 MJ/kg	Total collection method	28-d old broilers
	Linseed	24	–0·42 MJ/kg	Total collection method	29-d old broilers

[1]AME, Apparent metabolizable energy.

Table 8. Average feed intake of birds during the experimental period (g/bird)

Criteria	Basal diet	Diet $_{BSFL\ 5\%}$	Diet $_{BSFL\ 10\%}$	Diet $_{BSFL\ 15\%}$
Average feed intake (g/ bird) ± SEM	134.5±2.95	121.1±1.46	130.7±3.61	136.6±6.74
P-value		0.09		

SEM, Standard error of mean; BSFL, Black soldier fly larvae.

Table 9. Apparent metabolizable energy values of Black soldier fly larvae meal reported in the past research studies

Reference	Feed ingredient	Inclusion level (%)	AME value	Method	Bird type
Uushona (2015)	BSFL meal dried at 100 °C	50	14.8 MJ/kg	Marker method	43-d old, Cobb 500 broilers
	BSFL meal dried at 65 °C	50	17.4 MJ/kg	Marker method	43-d old, Cobb 500 broilers
	Defatted BSFL meal dried at 65 °C	40	16.5 MJ/kg	Marker method	43-d old, Cobb 500 broilers
Schiavone et al. (2017)	Partially defatted BSFL meal	250 g/kg (w/w)	16.3 MJ/kg DM	Marker method	Ross 308 male broilers
	Highly defatted BSFL meal	250 g/kg (w/w)	14.9 MJ/kg DM	Marker method	Ross 308 male broilers
Cockcroft (2018)	Dry-rendered BSFL larvae	50%	16.7MJ/kg	Total collection method	Cobb-500 broilers
	Extruded BSFL larvae	50%	8.84MJ/kg	Total collection method	Cobb-500 broilers
	Full-fat BSFL larvae	50%	15.8MJ/kg	Total collection method	Cobb-500 broilers

BSFL, Black soldier fly larvae; AME, Apparent metabolizable energy.

Furthermore, the effect of the experimental error on the estimated AME value decreases as the level of test material inclusion increases (Sibbald and Slinger, 1963). Moreover, at low inclusion levels, the test material may not be evenly mixed or sufficiently incorporated into the diet therefore may result inaccurate AME estimation if birds do selective intakes (Petersen et al., 1976). Therefore, it could possibly magnify the errors. The feed intake of birds is one of the vital factors which has a positive relationship when determining AME of a particular test ingredient (Sibbald, 1975; Farrell et al., 1991; Yaghobfar & Boldaji, 2002). Furthermore, the value for AME is also determined by endogenous energy loss (EEL) per unit of feed intake. Theoretically, as food intake decreases, the bird will force to meet more of its protein and energy needs through tissue catabolism, increasing endogenous energy output. Fasted birds therefore may have higher fecal and urinary energy losses than those birds full fed due to increased catabolism of tissue protein to provide energy for body maintenance. Uric acid, which is excreted as urine, is also a byproduct of protein catabolism (Sibbald, 1985). Though statistically not significant, in the present experiment the birds fed 5% BSFL meal indicated comparatively a lower feed intake as compared to those fed 15% inclusion level (Table 8). This might have an influence for a higher EEL resulting a negative AME value at 5% BSFL inclusion level.

The values reported in the past BSFL metabolic experiments are summarized in Table 9. Those results are in close agreement with the AME value obtained for 15% BSFL inclusion level (15.7 MJ/kg). Hence it is reasonable and noteworthy to consider 15.7 MJ/kg value as the AME of the BSFL full-fat meat under the conducted experimental conditions. Considering the plant-based AME experiments on white lupin (*Lupinus albus)* three cultivars namely, Ultra, Kiev mutant and Promore have resulted AME values of 8.05, 9.58 ad 9.68 MJ/kg DM, respectively) (Nalle et al., 2012). Interestingly, the present study demonstrated comparatively a higher AME value (15.7 MJ/kg) than the AME of plant-based protein ingredients in an economical manner.

The present AME study was conducted using Cobb 500 unsexed broilers aged between 25 to 28-days post hatch. Determination of AME of a particular feed does not depend only on the energy-gaining feed ingredients but also on the bird's health status, age, breed, feeding pattern, method of feeding assay, physiological conditions, housing conditions and environmental conditions (Härtel, 1986; Wu et al., 2020). By considering the effect of gender on AME, Nalle et al. (2012) has been proposed that the gender influences on digestive capabilities, gut structure, function, and metabolic activity of gut microflora. Furthermore, the AME value of a feed ingredient is known to be higher in adult chickens than in growing broilers (Sibbald and Wolynetz, 1985). Therefore, further studies are warranted to find out the AME of BSFL in the birds of the same sex.

Conclusion

The present study concluded that the full-fat BSFL meal is enriched with major nutrients, which could be substituted with conventional protein rich feed ingredients. Apparent metabolisable energy of full-fat BSFL estimated in the present study is 15.7 MJ/kg.

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Polygalacturonase Production by *Sarocladium strictum* T4 Isolate using Apricot Pulp as Substrate in Non-Sterile Culture Conditions

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ABSTRACT

In this study, 34 molds showing pectin degradation activity were isolated from the soil of orchards, by several tests. By using these isolates, pectinase group enzymes; studies on pectin lyase and polygalacturonase production were performed in the batch system and under non-sterile culture conditions. 5 isolates denoting polygalacturonase activity were coded as: T1, T2, T3, T4, T5 and the best polygalacturonase activity among these isolates was determined as 354.4 U/L in T4 isolate. Thus, optimization analyses continued by the use of this isolate. Initial apricot pulp concentration, temperature, pH and incubation period were tested as optimization parameters. The effects on enzyme activities were investigated by changing the initial apricot pulp concentration in the range of 5-100 (g/L), and in this regard, polygalacturonase activity was determined as 397.4 U/L at 50 g/L. In addition, the pH parameter was analyzed in each unit between pH = 3 – 8, and the temperature was tested by increasing 5 units in the range of 5-25°C. Consequently, the maximum polygalacturonase activity was determined as 405.7 U/L at pH 5 and 406.3 U/L at 15°C. Besides, the effect of the incubation period was studied within 1-5 days and the maximum polygalacturonase activity was determined as 429.0 U/L on the 4th day (after 96 hours). As a result, the above-mentioned T4 isolate, with which the optimization studies were conducted, was identified as *Sarocladium strictum* (Top ekinküfü) T4 by molecular methods.

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Introduction

Pectin is a carbohydrate compound from the pectic substance group with colloidal properties and is an additive providing benefits with its gel-forming ability. It is partially soluble in water. It is mostly found in foods, in the structure of vegetables and fruits, among the cells and in cell walls of all plantlets (Wang et al. 2014). It is critical to protect pectic compounds during the drying of fruits and vegetables. The pectin content of apricots is at the level of 1%. There are reports of losses in invert sugars such as galactose and arabinose in the pectin chain and a decrease in cell wall stiffness (Femenia 1998). Having great industrial importance, the enzymes that break down pectic substances are referred to as pectinolytic enzymes or pectinases (Fogarty and Kelly 1983; Saad et al. 2007). Research demonstrated a positive correlation between the polygalacturonase and pectin methyl esterase activity and the onset of softening in fruits such as apricot, papaya and mango (Roe and Bruemmer 1981; Femenia 1998). Pectinases are one of the enzymes that are mostly common in bacteria, fungi and plants. Pectinase production by filamentous fungi varies according to strain, composition of the growth medium and culture conditions (pH, temperature, aeration, shaking and incubation period (Souza et al. 2003). Having a large share in the world enzyme market, pectinase enzymes are often used in breeding flax and vegetable fibers, extraction of essential oils from the peels of vegetables and citrus fruits, pre-treatment of pectic wastewater, fermentation of coffee and tea, producing poultry feed, distilling plant viruses, extracting fruit juice and the decantation process (Hoondal et al. 2002; Saito et al. 2004; Uzuner and Çekmecelioğlu 2016). The pectinase enzyme has a 25% share in the global sales of food enzymes, and this rate is expected to increase over time with the discovery of new areas to use the enzyme (Jayani et al. 2005). This enzyme is most commonly obtained commercially from microbial pathways and especially from molds. Microorganisms are the most suitable organisms for enzyme production and currently represent 90% of the total market (Sanchez and Demain 2011).

The last few years have witnessed prominence in research on enzymes that show activity at low temperatures in cold-adaptive organisms. Among the main reasons for this, it is stated that cold-adaptive enzymes have a higher specific activity compared to mesophilous ones at low and moderate temperatures (Antranikian et al. 2005). A reason why they come to the fore is that these enzymes help achieve significant energy savings in practice (Morita et al. 1998).

Today, by-products of the fruit processing industry pose an important waste problem. There is a growing interest in these wastes, though, for the high nutritional values contained in their waste components and for their recyclability. In addition, these wastes can be used as food additives and supplements. Furthermore, these waste products can be used as a substrate in the prepared media. As a matter of fact, more and more international studies have been focusing on issues such as clean energy generation, waste use and recycling.

This study used the *Sarocladium strictum* (Top ekinküfü) (Güner et al.2020) T_4 isolate and apricot pulp as a substrate for the production of polygalacturonase. To save time, workload and electricity, the study was conducted in a non-sterile medium and under non-sterile culture conditions.

Materials and Methods

Preparing suitable growth medium and isolating active pectinase-producing microorganisms in cold

10-20 different soil samples were taken from the orchards within the borders of Erzurum province. Dilution series of 10^{-1},10^{-2}, $l0^{-3}$ and 10^{-4} were prepared with 0.9% sterile physiological saline (FTS). From each of the samples, 5 g\L Pectin, 1 g\L; KH_2PO_4, 1 g\L; $(NH_4)_2S0_4$, 0.75g\L; $MgSO_4$, 0.015g\L; $FeSO_4$, 0.15g\L; $CaCl_2$, 0.25g\L; NaH_2PO_4, 20 g\L petri dishes were prepared in accordance with the Agar medium. By the help of a sterile 0.1 swab, these were spread in the petri dishes, and left for incubation in the refrigerant at +4°C. Apricot pulp was used as a solid substrate for the development of pectinase-producing microorganisms. Apricot pulp was supplied as a by-product from a fruit juice factory in the Aegean Region and dried for 24 hours in an incubator at 60°C. It was then ground and used in the analyses.

Identifying Pectin Lyase and Polygalacturonase Activities of the Isolates

For the polygalacturonase activity determined by editing the method in the study by Patil et al. (2006); 0.7 mL Acetate Buffer (pH 5.5 and 0.1 M), 2 mL 0.5% pectin solution and 1 ml enzyme sample were incubated at 45°C for 30 minutes. The reducing sugars were determined by the dinitro salicylic acid (DNS) method, which used galacturonic acid as reference. The DNS solution was prepared by mixing 1% DNS, 0.2% phenol, 0.05% sodium sulfite, 1% sodium hydroxide, 30% sodium potassium tartrate and by adding pure water to reach the amount of 100 ml. The mixture was incubated at 45°C for 30 minutes, and then 0.1 mL was taken from it. Later, 1.9 mL distilled water and 2 mL DNS were added to it. The mixture was kept in a boiling water bath at 90°C for 20 minutes and the final volume was completed to 15 ml. After cooling, the absorbance was read against the blind solution at a wavelength of 550 nm. The amounts of galacturonic acid were calculated using a calibration curve. Figure 1 shows the calibration graph obtained by reading the absorbance of galacturonic acid solutions prepared at certain concentrations from a galacturonic acid solution of 50 mmol/L (Tepe, 2012) at 550 nm.

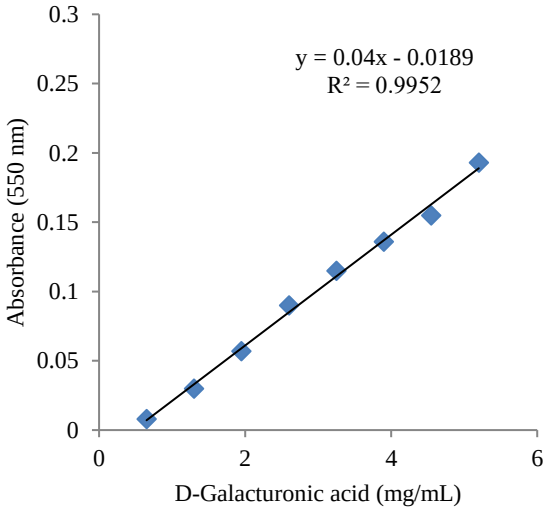

Figure 1. D-galacturonic acid standard curve graph

Calculation of Enzyme Activity

By applying the absorption values at 550 nm to the d-galacturonic acid regression equation, the amount of d-galacturonic acid released by the enzyme in one minute was determined in terms of µmol/minute (Debinga et al. 2005). One unit of enzyme activity is the amount of enzyme that forms 1µmol of D-galacturonic acid from pectin in one minute under standard conditions. The amount of reducing sugar found using the standard D-galacturonic acid curve was calculated by adding it to the equation given below (U/mL):

Total Unit = mg/ml reducing sugar × 100/t × MG

mg/mL: The amount of D-galacturonic acid corresponding to the absorbance of the samples
t : Incubation period (min)
MG : Molecular weight of the released D-galacturonic acid

The fungal biomass was found by measuring the amount of N-acetyl glucosamine released by acid hydrolysis from the fungal kit in the solid fraction (Nimnoi and Lumyong 2011; Velmurugan et al. 2011). For this, a sample (10mk) was taken from the culture and centrifuged at 5000 rpm for 10 minutes. Then, 1 ml concentrated H2S04 (sulfuric acid), and acetylacetone reagent (1 mL) were added respectively to the resulting 0.5g solid fraction. This mixture was incubated in a boiling water bath for 20 minutes. Later, it was cooled at room temperature. Followed by this step, 6 mL Ethanol and 1 mL Ehrlich reagent were added to the mixture. After this mixture was incubated at 65°C for 10 minutes and cooled again, its optical density was found as 530 nm. N-acetyl glucosamine (Sigma-Aldrich) was used as standard.

The cell biomass (g/L) was calculated using the formula indicated below:

Cell biomass (g/L) = (N-acetyl glucosamine (g/L).100)/8.3

The degree of contamination that was likely to occur in the culture medium during the experiments were shown according to microscopic examinations as follows: no contamination was indicated by (-), low contamination by (+), moderate contamination by (++) and high contamination by (+++). The conditions under which less or no contamination was observed and maximum enzyme activity was achieved were identified and used in the next step.

Results and Discussion

Significant polygalacturonase activity was obtained in only 5 of the 34 isolates tested in the study. Erzurum Technical University's Department of Molecular Biology and Genetics made the molecular diagnosis of the T4 isolate, which showed the best polygalacturonase activity among all the isolates used in the study, and identified it as *Sarocladium strictum* T4.

Optimization of polygalacturonase activity with the T4 isolate

The effect of substrate amount on enzyme activity

Polygalacturonase activities in different concentrations of apricot pulp used instead of pectin were identified according to absorbance values of 550 nm after 72 hours at 15°C, pH 6 and 170 rpm mixing speed. As the initial substrate concentration increased, enzyme production increased, and at the point where the number of enzyme molecules was low relative to substrate molecules, the increase in substrate concentration with the formation of an enzyme-substrate complex did not affect the speed. No substrate inhibition was observed in the analyzed range. The maximum polygalacturonase activity was found as 397.4 U/L at 50 g/L and was used as the initial substrate amount in subsequent experiments. The results manifest that the presence of pectic substances in the fermentation medium affects enzyme production. Pectin does not cause inhibition on microorganism reproduction or enzyme activity. In the same vein, Abbasi and Fazaelipoor (2010) found that 50 g/L pectin concentration does not cause inhibition on polygalacturonase activity.

The effect of pH on enzyme activity

The activity of the polygalacturonase enzyme between pH 3-8 at every other unit was found according to the absorbance values of 50g/L substrate (apricot pulp) at 15°C and 170 rpm mixing speed, at 550 nm after 72 hours, and the optimum pH, at which it was active, was determined to be 5 and the enzyme activity was 405.7 U/L. It is also stated in the literature that the optimum pH for the reproduction of most fungi and the production of pectinase group enzymes varies in the 5.0-7.0 range.

The effect of temperature on enzyme activity

Under standard test conditions with 5-25°C temperature and fixing at PH 5, the effect of temperature on pectinase activity was tested by 50g/L substrate (apricot pulp) and mixing at a speed of 170 rpm tested. According to the absorbance values at 550 nm after 72 hours, the maximum PG activity was reached at 15°C and was determined as 406.3 U/L. The fact that *Sarocladium strictum* also showed polygalacturonase activity at low temperature indicates that the enzyme is cold-adaptive.

Table 1. Polygalacturonase activity of isolates (Duncan test)

Isolates	PG Activity (U/L) Biomass (g/L)
T1	301.0^{b} 6.590^{c}
T2	284.4^{c} 6.867^{b}
T3	85.20^{e} 4.373^{e}
T4	354.4^{a} 7.349^{a}
T5	188.0^{d} 5.181^{d}

*The difference between the averages denoted with the same letters in the same column is not statistically significant (P<0.05).

Table 2. The effect of the initial substrate concentration on PG activity (Duncan test)

Initial Substrate Concentration (Apricot pulp) (g/l)	PG Activity Biomass Contamination (U/L) (g/L)
5	350.0^{d} 7.351^{h} ++
10	360.8^{cd} 7.599^{g} ++
20	367.3^{bcd} 7.768^{f} ++
30	378.7^{bcd} 7.926^{e} ++
40	386.4^{bc} 8.168^{d} ++
50	397.4^{ab} 8.314^{a} ++
60	396.4^{ab} 8.283^{b} ++
70	395.7^{ab} 8.273^{b} ++
80	395.1^{ab} 8.241^{c} ++
90	394.9^{ab} 8.274^{b} ++
100	394.7^{ab} 8.276^{b} ++

*The difference between the averages denoted with the same letters in the same column is not statistically significant (P<0.05).

Table 3. The effect of pH on enzyme activity (Duncan test).

pH	PG Activity Biomass Contamination (U/L) (g/L)
3	17.00^{f} 1.349^{e} -
4	108.0^{e} 2.636^{d} -
5	405.7^{a} 8.331^{a} -
6	397.0^{b} 8.302^{a} ++
7	297.9^{c} 6.987^{b} +++
8	183.1^{d} 3.899^{c} +++

*The difference between the averages denoted with the same letters in the same column is not statistically significant (P<0.05).

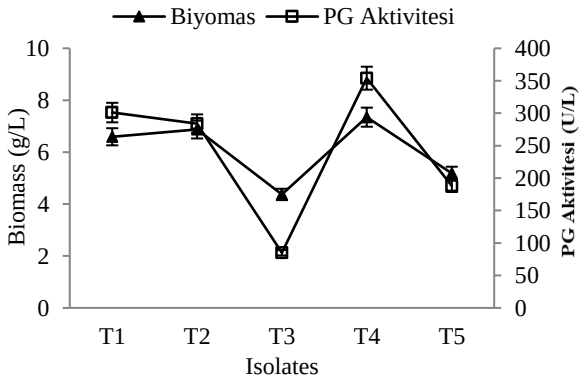

Figure 2. The polygalacturonase activity of the isolates used (Culture conditions: PG activity after 72 hours at 15°C, 5g/L KP, pH 6 and 170 rpm shaking incubator)

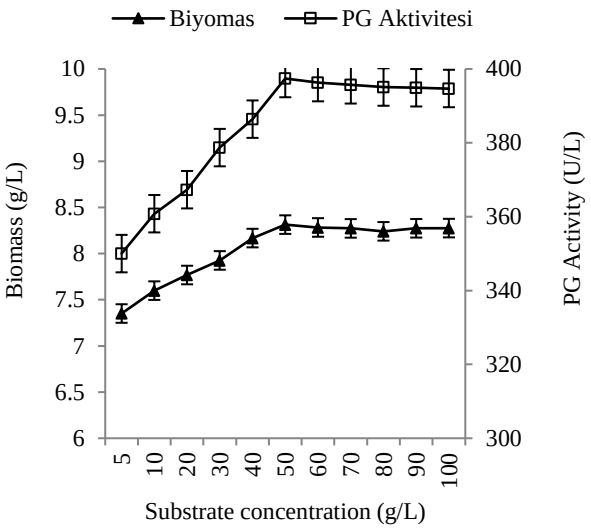

Figure 3. The effect of substrate amount on enzyme activity

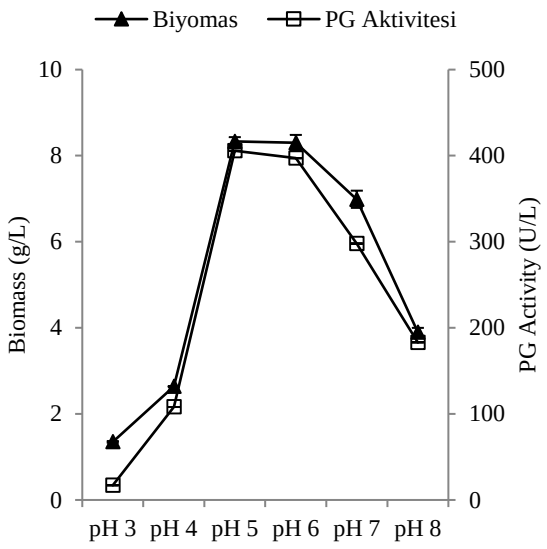

Figure 4. The effect of pH on enzyme activity

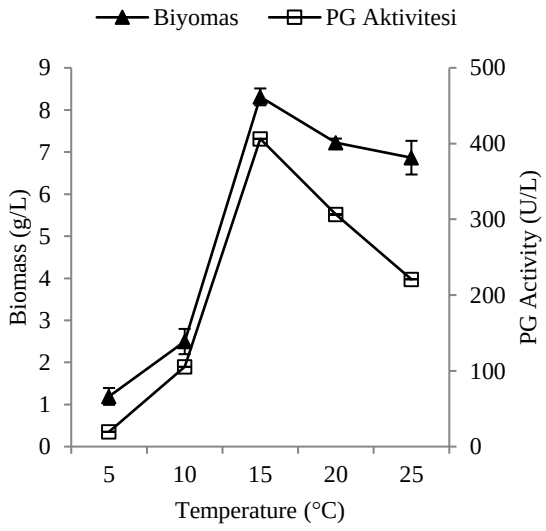

Figure 5. The effect of temperature on enzyme activity

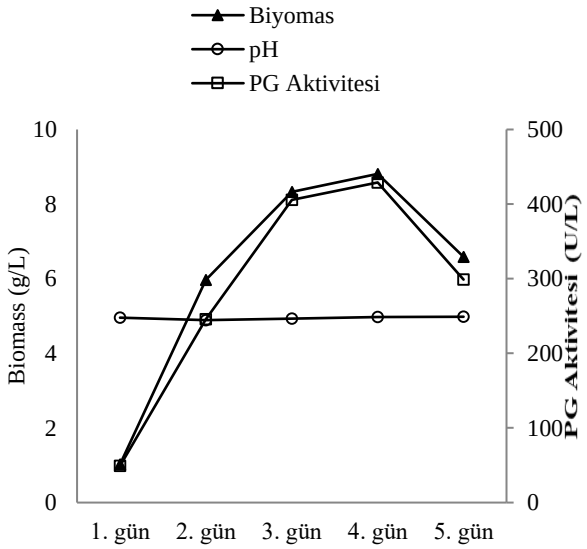

Figure 6. The effect of incubation period on enzyme activity

Table 4. The effect of temperature on enzyme activity**

Temperature (°C)	PG Activity Biomass Contamination (U/L) (g/L)
5	19.50^{e} 1.192^{e} -
10	105.3^{d} 2.496^{d} -
15	406.3^{a} 8.310^{a} -
20	306.4^{b} 7.219^{b} -
25	220.9^{c} 6.865^{c} -

*The difference between the averages denoted with the same letters in the same column is not statistically significant (P<0.05). **(Duncan test)

Table 5. The effect of incubation period on enzyme activity**

Incubation period (hour)	PG Activity Biomass Contamination (U/L) (g/L)
24	49.20^{e} 1.024^{e} -
48	245.7^{d} 5.970^{d} -
72	405.5^{b} 8.332^{b} -
96	429.0^{a} 8.810^{a} -
120	298.7^{c} 6.582^{c} -

*The difference between the averages denoted with the same letters in the same column is not statistically significant (P<0.05). **(Duncan test)

Considering this property, it can be said that it has a high potential to be used in especially the detergent industry where low temperature application is targeted.

The effect of incubation period on enzyme activity

To determine the incubation period that the enzyme can show maximum activity, the activities of how 50g/L substrate (apricot pulp) were evaluated for five days at 15°C, pH 5 and at a mixing speed of 170 rpm at 550 nm absorbance values. The maximum polygalacturonase activity was found as 429,0 U/L on the 4th day (after 96 hours). After this time, a decrease in PG activity was observed in the media. In addition, the ambient pH was checked during this 5-day period, and no significant changes were observed between the measured pH values.

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Investigation of Leaf Gas Exchange Parameters of Several Chestnut Population Seedlings at the End of the Growing Season

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ABSTRACT

Changes in temperature and precipitation due to global climate change negatively affect plant species' growth, development, and adaptation to new places. However, genetic structure is the most critical criterion for determining a species's potential to adapt to changing environmental conditions. Monitoring gas exchange parameters in plants is the simplest way to monitor physiological changes in plants under changing environmental factors. Among species, the Anatolian chestnut (*Castanea sativa*) is native and economically important tree species (fruit and wood production). It is naturally distributed from the north side of Turkey, Marmara, and Western Anatolia. However, the Anatolian chestnut is one of the most affected tree species by global climate change. In this study, numerous Anatolian chestnut populations (3 years old) were used to determine leaf gas exchange parameters at the end of the growing season in Düzce. Stomatal conductance (*gs*), transpiration rate (*E*), net photosynthetic rate (*Anet*), and other parameters were measured. As a result, the leaf gas exchange parameters of chestnut populations changed significantly based on the populations. Marigoule population seedlings had 2-fold *Anet* values compared to the Ibradı population. Regarding *gs*, the differences between populations (Erfelek and Ibradı) changed approximately 2.5 folds and the differences (Erfelek and Ibradı) increased more than 3 folds in terms of *E* values. It can be said that Marigoule and Erfelek populations can adapt more to Düzce climate conditions than other populations. In contrast, Ibradi population seedlings have a low adaptation mechanism in terms of gas exchange traits.

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Introduction

Temperature and precipitation are the two main drivers for plant distribution around the world. With the Industrial Revolution, people have been heavily using natural resources, such as coal, gases and some other elements, resulting in increased air pollution (Çobanoğlu et al., 2022; Isınkaralar et al., 2022; Key et al., 2022; Koç et al., 2022), water contamination (Demir et al., 2021; Kutlu and Mutlu, 2021; Tokatli et al., 2021; Mutlu and Uncumusaoglu, 2022) and temperature on a regional and global scale (IPCC, 2014), and therefore a change occurs in the climate (Koç, 2022a). The temperature has risen around 1 ºC in the previous century (IPCC, 2014), and the mean annual air temperature may increase by 2.5 and 5.4 °C by 2050 and 2100, respectively, in the world (IPCC, 2014), and Türkiye (Cantürk and Kulaç, 2021). The temperature increases cause some adverse effects on plant species, such as drought (Seleiman et al., 2021; McDowell et al., 2022; Koç, 2022a) and cold stress (Yildiz e al., 2014; Raza et al., 2019; Chaudhry and Sidhu, 2021), which negatively affect tree growth, development, and physiology (Koç and Nzokou, 2022; Koç and Nzokou, 2023). All these changes are related to the genetic variation of plants.

Genetic diversity is the fundamental component that enables species to survive in changing ecosystems. In addition, genetic diversity is the most important criterion that determines the potential of a species to adapt to different environmental conditions (Sevik et al. 2010). This situation has become much more important for plants, especially with global climate change and global warming, the effects of which are increasing daily. Therefore, it is crucial to determine how different species populations behave under these changing environmental stress factors (Koç, 2022a). Identifying the most resistant populations or adaptable to these changing stress factors is vital and encourages their use. Genetic variation may play an essential role during the photosynthetic process, especially in diverse environments.

As a physiological parameter of plants, leaf photosynthetic gas exchange directly affects plant growth, and therefore scientists have been aware of this subject recently. However, similar plant species differ even under the same environmental conditions (Bhattacharjee and Saha, 2014; Koç and Nzokou, 2022). Species have various abilities to respond to environmental fluctuations (Allen et al. 2010; Koç et al., 2022). All the response mechanism to environmental alterations is linked to species' genetic structure (Koç and Nzokou, 2022). Plant species' morphological, anatomical, and photosynthetic traits are formed by the interplay of genetic codes and environmental situations (Ozel et al. 2021).

Photosynthetic gas exchange from leaves, using the LI-COR photosynthesis method (LI-COR Biosciences, Lincoln, NE, USA), is one of the common, reliable, and robust methods to determine plant status instantly. The LI-COR 6800 is worked based on a unit area on the plant leaf and a closed system that maintains IRGA, relative humidity, light intensity, and CO_2. The portable LI-COR 6800 is the latest version that provides a series of measurements, such as transpiration rate, net assimilation (photosynthetic) rate, stomatal conductance, intrinsic CO_2 concentration, and many more. Using some of these measurements, water use and intrinsic water use efficiency are also calculated. Water use efficiency has reflected the percentage of net photosynthetic ratio and transpiration, while intrinsic water use efficiency indicates the percentage of net photosynthetic ratio and stomatal conductance (Lambers et al. 2008; Koç, 2022a).

Among broad-leaf species, the Anatolian chestnut (*Castanea sativa*) is native tree species in Turkey and is distributed from the north side of Turkey from Bulgaria to the Caucasus, besides Marmara and Western Anatolian provinces (Orman Genel Müdürlüğü (OGM), 2013). Anatolian chestnut is the main tree species and is economically significant in Turkish forestry due to its fruit and wood production. It has a smooth and plump trunk that has been used in many areas, such as decorative, furniture, and other construction purposes (Conedera et al. 2004; Kakava et al. 2018; Mirela, 2020). Türkiye has around 39-40 thousand ha chestnut production areas, which ranks third in the world and earns more than $40 million from its export (about 60 thousand tons) worldwide in 2018 (Food and Agriculture Organization of the United State (FAO), 2019).

Türkiye is among the "countries at risk" against climate change (UNDP, 2019), and the most adverse impact of global climate change will manifest itself in the Mediterranean region in the form of increased temperatures and decreased precipitation (Giorgi and Lionello, 2008). It is estimated that these changes will significantly affect chestnut forests as in many species (Talu et al. 2011). Species that do not have an adaptation mechanism to these rapid climate changes will suffer the most (Lindner et al. 2010), and therefore some local populations will be in danger of extinction (Keenan, 2012).

For these reasons, various studies have been conducted on the possible effects of climate change on the adaptation of different species populations and plant species in different ecosystems. Most such studies harm plant species; however, plant species' have some mechanisms against climatic changes in the regions that affect plants' morphological and physiological parameters. The most harmless method to determine the changes in these physiological structures is the examination of the leaf gas exchange parameters of the plants. Therefore, seeds were collected from chestnut populations adapted to different regions of Turkey, seedlings were grown, and the differences between gas exchange parameters of these populations in the same environment (Düzce province) were tried to be determined.

Material and Method

Seed Collection, Sowing, and Containerization Substrates

The chestnut seeds were collected from at least 10 different trees in each population from September to October 2017. The information about populations is given in Table 1. The seeds were subjected to wet, cold stratification for about 28-45 days for germination, and then germinated seeds were sown into 18x25 cm polyethylene plastic bag where the potting mix consisted of peat moss, forest soil, and perlite (1:1:1 volume). The seedlings were placed outside on the concrete floor and watered once every week when it was no rain for the following years. In the warmer summer (July-September), the top was covered with a shade of material to prevent sunburn on leaves and water on hot days. This experiment was conducted at the Düzce University Forestry Department nursery in 2021. The stem height and diameter growth (± S.D.) for each chestnut population during the measurement period are given in Table 1.

Table 1. The origins of chestnut populations and their seedling growth parameters

Codes	Origin	Height (cm)	Diameter (mm)
IBR	Ibradı (Akseki-Antalya)	90.50 ± 19.21	16.20 ± 3.73
OVA	Ovacık (İzmir)	87.20 ± 19.92	12.95 ± 2.95
DSA	Deli Sarnıç (Antalya)	96.70 ± 22.43	11.18 ± 2.17
SEL	Selge (Antalya)	84.50 ± 22.23	10.76 ± 2.20
BUR	Bursa	106.50 ± 19.57	14.22 ± 2.41
DKAB	Kabalak (Düzce)	98.70 ± 24.29	11.76 ± 1.54
ERF	Erfelek (Sinop)	79.10 ± 25.54	11.30 ± 2.28
AOM	Oluk Mahallesi (Antalya)	85.00 ± 24.32	11.81 ± 2.22
DKAP	Kaplandağı (Düzce)	95.10 ± 20.99	11.66 ± 1.59
MAR	Marigoule	60.50 ± 15.76	8.07 ± 0.82

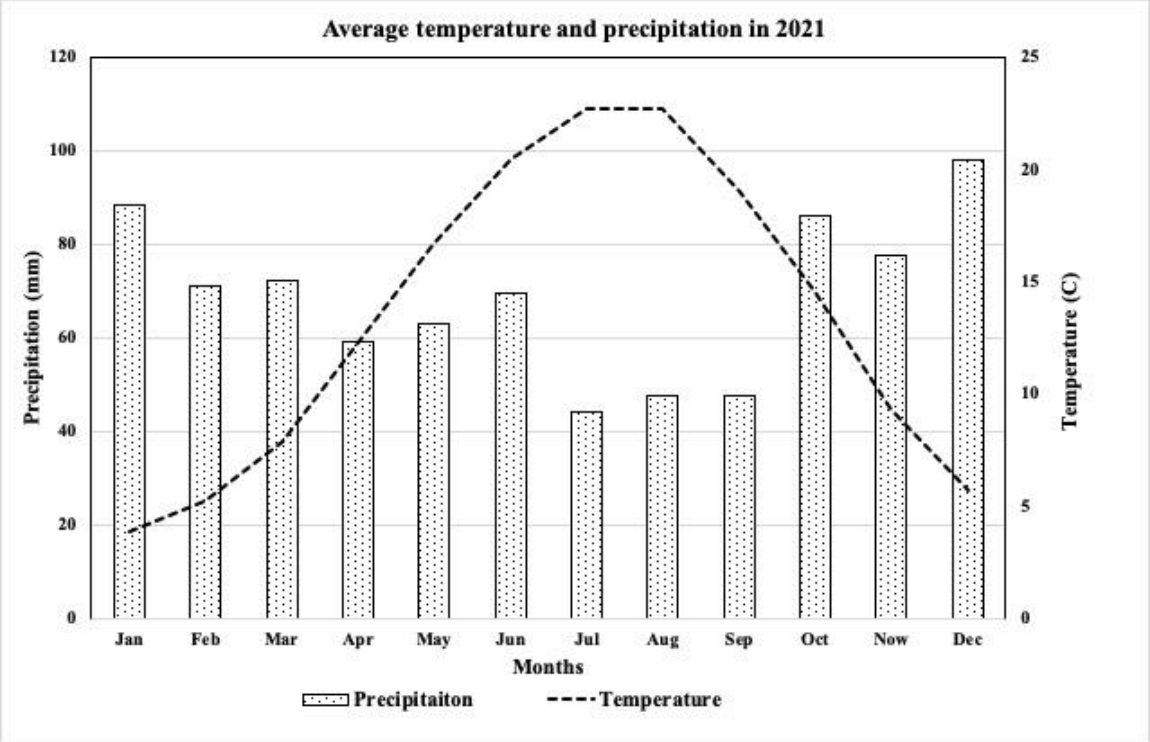

Figure 1. The mean monthly temperature and precipitation

The seeds were subjected to wet, cold stratification for about 28-45 days for germination, and then germinated seeds were sown into 18x25 cm polyethylene plastic bag where the potting mix consisted of peat moss, forest soil, and perlite (1:1:1 volume). The seedlings were placed outside on the concrete floor and watered once every week when it was no rain for the following years. In the warmer summer (July-September), the top was covered with a shade of material to prevent sunburn on leaves and water on hot days. This experiment was conducted at the Düzce University Forestry Department nursery in 2021. The stem height and diameter growth (± S.D.) for each chestnut population during the measurement period are given in Table 1.

Temperature and Precipitation

The meteorological data (temperature and precipitation) was obtained from Düzce Meteorological Station for 2021. The city's average monthly temperature and precipitation are given in Figure 1.

Growth Parameters and Gas Exchange Measurements

The height growth was measured with a wooden tape measure, while diameter growth was measured using a caliper in 10 seedlings for each population.

The gas exchange parameters measurement was performed on eight different seedlings (a single reading was taken from each seedling) from each population using a broad leaf chamber (9 cm^2) of LI-COR (LI-6800, Lincoln, NE, USA) with an attached light source (6800-02 – red/blue/light). The calibration was done as recommended by the producer. Then, the airflow rate, PPFD (photosynthesis photon flux density), and reference CO_2 were set and held automatically at 500 µmol s^{-1}, 1500 µmol s^{-1}, and 400 µmol mol^{-1} s^{-1}, respectively. The measurement was taken on September 29, 2021.

The stomatal conductance (*gs*, µmol H_2O m^{-2} s^{-1}), transpiration rate (*E*, mmol m^{-2} s^{-1}), net photosynthetic rate (*Anet*, µmol m^{-2} s^{-1}), and intercellular CO_2 (*Ci*) within gas exchange parameters were directly measured when the intercellular CO_2 to ambient CO_2 (*Ci*/*Ca*), intrinsic water use efficiency (*iWUE=Anet/gs*), water use efficiency (*WUE=Anet/E*), parameters were calculated.

Data Analysis

This experimental design was a complete randomized consisting of 10 populations, and each population had eight seedlings (replication) subjected to gas exchange measurement. Data analysis of all gas exchange parameters was done using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). PROC MIXED function was used to perform analysis of variance (ANOVA), and mean separation of populations was done using Tukey's adjustment.

Result

Height Growth (HG) and Diameter Growth (DG)

The growth parameters (HG and DG) of populations are given in Table 1. The populations of Bursa seedlings had the highest HG (106.50 ±19.57 cm), followed by the Kabalak population (98.70 ±24.29 cm), while Mariguole population seedlings had the lowest HG (60.50 ±15.76 cm). The other populations averaged HG values are close to each other. The highest DG was observed in the seedlings of the Ibradı population (16.20 ±3.73 mm), followed by the Bursa population (14.22 ±2.41 mm) and Ovacık population (12.95 ±2.95 mm), while the seedlings of the Mariguole population s had the lowest DG (8.07 ±0.82 mm). The rest of the population seedlings means DG is almost the same.

Table 2. F-values of ANOVA for leaf gas exchange traits of chestnut populations

Source of variation	df	Anet	gs	E	iWUE	WUE	Ci	Ci/Ca
Population	9	6.25***	4.75 ***	12.61 ***	8.92***	26.28***	6.27***	6.40***

*** P≤0.0001. Df = Degrees of Freedom.

Table 3. The Average Values of Gas Exchange Parameters among Chestnut Populations

Population	Anet	gs	E	iWUE	WUE	Ci	Ci/Ca
AOM	6.08 bc	0.051 abc	0.0017 bc	113.62 d	3350.37c	188.17 a	0.485 a
BUR	8.50 ab	0.068 ab	0.0020 ab	126.81 cd	4131.22 c	164.04 ab	0.430 ab
DKAB	6.21 bc	0.056 abc	0.0019 abc	114.8 d	3469.88 c	186.64 a	0.484 a
DKAP	6.47 bc	0.049 abc	0.0017 bcd	146.97 bcd	4135.37 c	135.16 abcd	0.350 abc
DSA	7.37 abc	0.048 abc	0.0012 cde	155.54 abc	5937.99 b	123.89 abcd	0.320 abc
ERF	8.79 ab	0.075 a	0.0024 a	126.6 cd	3893.91 c	163.00 abc	0.425 ab
IBR	4.71 c	0.029 c	0.0007 e	175.78 ab	6715.03 ab	98.02 cd	0.251 c
MAR	10.07 a	0.070 ab	0.0016 bcd	143.58 bcd	6238.45 ab	138.30 abcd	0.363 abc
OVA	7.64 abc	0.043 bc	0.0011 de	187.46 a	7538.61 a	84.94 d	0.220 c
SEL	8.80 ab	0.054 abc	0.0016 bcd	157.4 abc	5568.49 b	116.98 bcd	0.305 bc

Note: Different letters, such as a, b, c means that the parameters of gas exchange significantly (p<0.05) differed among chestnut populations.

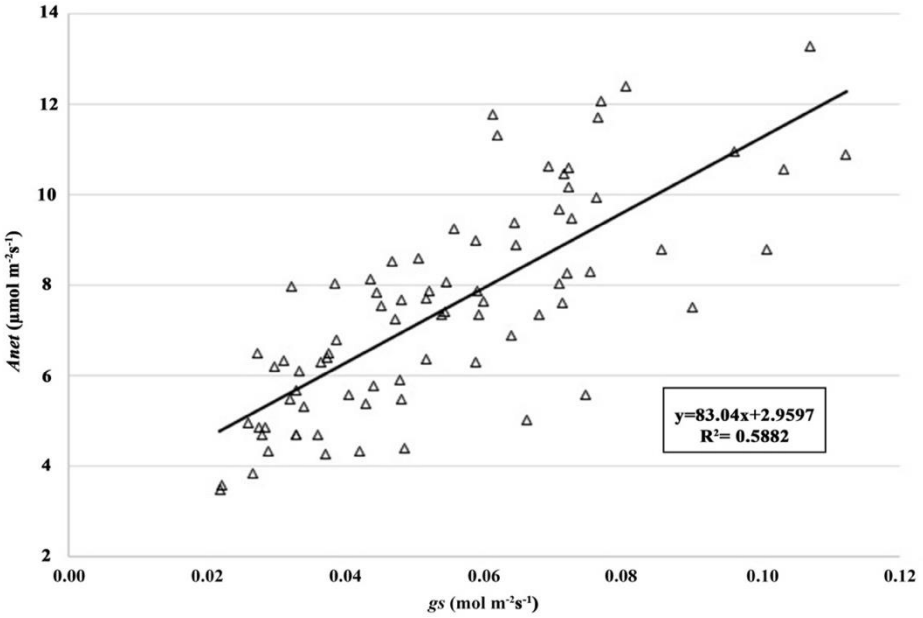

Figure 2. Relationship between gs and Anet within chestnut population

Leaf Gas Exchange Measurement

The ANOVA of leaf gas exchange measurements, such as *Anet*, *E*, *gs*, *WUE*, *iWUE*, *Ci*, *and Ci/Ca* among populations, are presented in Table 2. Chestnut population statistically differed (P≤0.0001) for each gas exchange parameter (Table 2). The averages and Tukey's test results of gas exchange parameters of chestnut populations are presented in Table 3.

When the chestnut populations are evaluated in terms of *Anet* amounts, the seedlings of the MAR population have the highest *Anet* amount. At the same time, the lowest average *Anet* value is found in the IBR population, followed by ERF and BUR populations (Table 3).

When the chestnut populations are evaluated in terms of *gs*, the seedlings of the ERF population have the highest average *gs* value, followed by MAR and BUR populations. The lowest *gs* values are determined in chestnut seedlings belonging to IBR populations (Table 3).

If we evaluate the chestnut populations in terms of *E*, the seedlings of the ERF population have the highest *E* values, followed by the BUR population. In contrast, the lowest *E* value is seen in the seedlings of the IBR population, followed by the seedlings of the OVA population (Table 3).

When the chestnut populations are evaluated in terms of *iWUE* amounts, the highest values are found in the OVA population seedlings, followed by the IBR population. The lowest *iWUE* values were determined in the AOM population, followed by the DKAB and BUR populations (Table 3).

When the chestnut populations are evaluated in terms of *WUE* amounts, the seedlings of the OVA population showed the highest value, followed by the group formed by the IBR, MAR, and SEL populations. The lowest *WUE* values were observed in a group consisting of AOM, DKAB, ERF, BUR, and DKAP populations (Table 3).

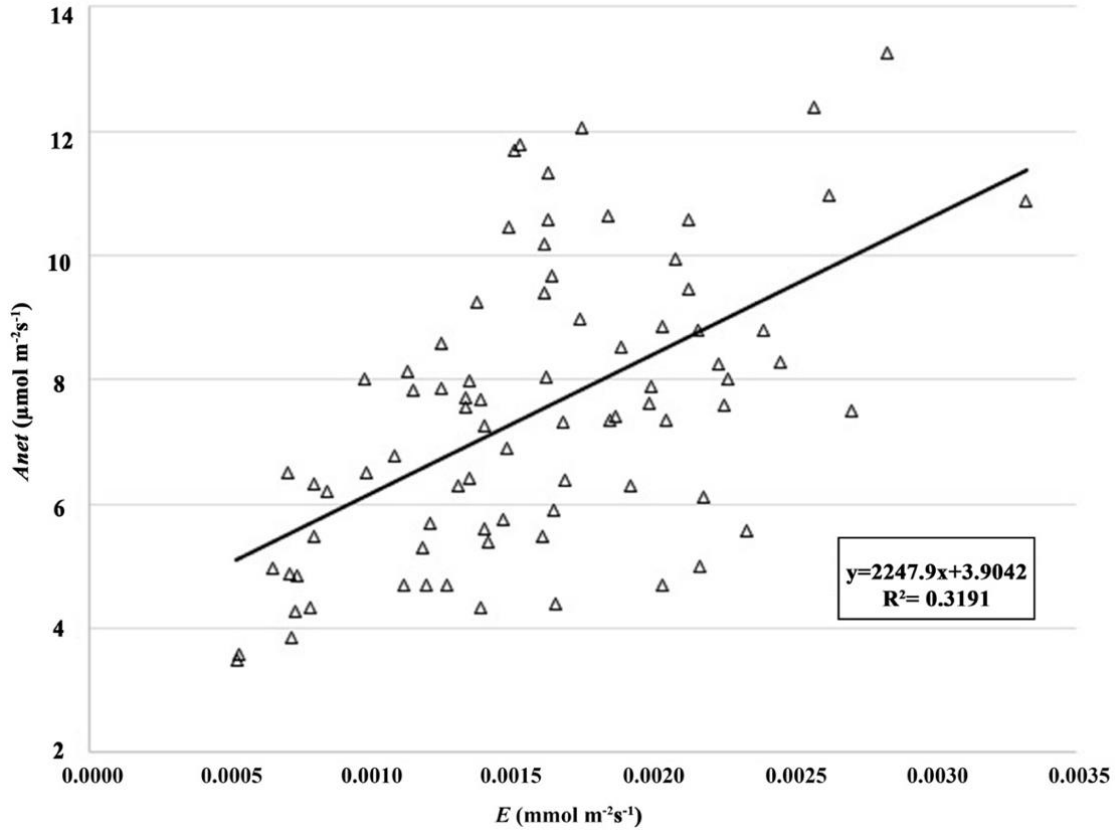

Figure 3. Relationship between E and Anet within chestnut population

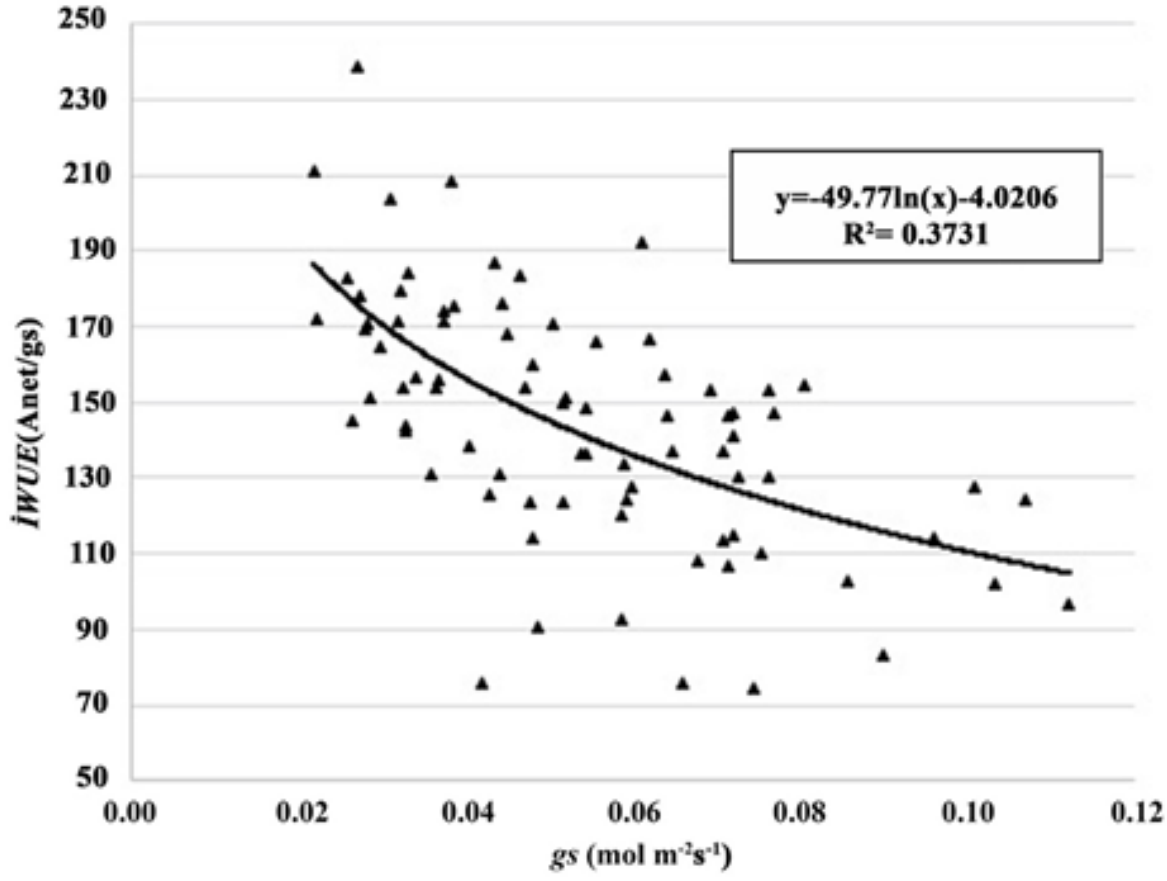

Figure 4. Relationship between gs and iWUE within chestnut population

When Ci values of chestnut populations were examined, AOM and DKAB populations had the highest values, whereas OVA and IBR showed the minimum *Ci* values (Table 3). When the *Ci/Ca* values of the chestnut populations are examined, the seedlings of the AOM and DKAB populations have the highest *Ci/Ca* values. In contrast, the OVA and IBR populations have the lowest values (Table 3).

In the current study, *Anet* correlated with *gs* that increasing stomatal conductance increased *Anet* in chestnut populations (Figure 2). However, there was not a strong correlation between *Anet* and *E* in the seedlings of chestnut populations (Figure 3). There was a negative correlation between *iWUE* and *gs*, which was not robust (Figure 4).

Discussion and Conclusion

As a result of the study, it was determined that the leaf gas exchange parameters changed significantly based on the population, and even this change was determined to be approximately 2 times higher in terms of the *Anet* values amount among some populations (MAR and IBR). Regarding *gs*, the differences between populations (ERF and IBR) changed approximately 2.5 times, and the differences (ERF and IBR) increased more than 3 times in terms of *E* values. These differences in *iWUE* and *Ci/Ca* values were determined as 1.5 (OVA and DKAB) and 2 (AOM and OVA) folds, respectively. Reduced water content in the soil causes a decline in plant organs' water potential, causing a reduction in *gs*, *Anet*, and *E*. Similar results were observed in *Acer negundo* and *Acer pseudoplatanus* (Koç, 2022a) and some conifer species seedlings (Koç 2021; Koç and Nzokou, 2022). A decline in *gs* caused a reduction in *Anet* and *E*, which led to plants having different *WUE* and *iWUE* traits. The different traits may play an essential role in plant growth and development (Xu et al. 2020).

Today, global climate change is one of the most critical glitches on a global scale, and this problem is considered irreversible (Varol et al. 2021; Tekin et al. 2022). It is estimated that global climate change will directly or indirectly affect all living things and ecosystems (Varol et al. 2022; Varol et al. 2022). Because the phenotypic features of all living things are shaped under the influence of genetic structure and environmental factors, the most apparent effects of global climate change will affect the climate parameters (Koç, 2022a; Yayla, 2022). It is estimated that the main effect of global climate change on climate parameters will manifest itself in the form of precipitation regime and temperature increase (Koç, 2022b; Koç, 2022c).

In this process, it is stated that factors such as fertilization deficiency, especially in marginal regions (Shults et al., 2018), increased temperature, more prolonged summer drought, and increased UV-B will significantly affect plant growth (Cantürk and Kulaç, 2021; Ozel et al. 2021). Because these factors, especially drought, are among the most critical stress facets affecting the development and growth of plants (Seleiman et al. 2021; Chaudhry and Sidhu, 2021; Koç, 2022a; Koç et al., 2022).

Summer temperatures and drought that will be prolonged due to global climate change will affect plant growth and may cause significant species and population losses (Varol et al. 2021). However, it is predicted that these impacts will have different levels of effects on a species basis, and while some species have significantly narrowed their distribution areas, the appropriate distribution areas of some species will increase (Dyderski et al. 2018; Ning et al. 2021).

It is estimated that species resistant to drought conditions will be less affected in this process. Studies reveal that the drought tolerance of different species differs significantly (Sevik and Cetin, 2015; Yigit et al. 2016). In addition, it has been determined that different populations of the same species have different levels of drought tolerance (Sevik and Erturk, 2015; Koç and Nzokou, 2022). Therefore, less resistant species and population losses are inevitable due to the effects of global climate change. In order to minimize these losses, necessary measures should be taken without delay.

In order to diminish possible species and population losses in the global climate change process, it is necessary to determine the climatic changes that will occur in advance. Studies on the subject it has been tried to determine both the change of climate parameters (Koç, 2022b; Koç, 2022c) and how this change will change the appropriate distribution areas of various species (Canturk and Kulaç, 2021; Tekin et al. 2022). It is essential to continue and diversify these studies to determine the climatic changes that may occur, which species will be affected by these changes, and to what extent.

Suggestions

In order to diminish the outcomes of climate change globally, especially species and population losses, these effects must be determined in advance. For this reason, projected model studies on the subject, determining the variables affecting this process, and determining possible changes in advance are priority issues.

In the next stage, to prevent deforestation, it is recommended to determine the most drought-resistant species and plant these species in appropriate areas, at least to include them in the mixture of the stands. Thus, although species and population losses are experienced in forested areas, at least the effects of deforestation and related erosion, loss of water resources, and large-scale loss of fauna can be reduced. Drought-resistant species in landscaping in urban areas are critical in preventing plant loss and saving water use.

One of the most critical measures that can be taken on the subject is the use of drought-resistant plant origins. Determining and using drought-resistant origins in the forest, agriculture, and landscape studies is very important in adapting to global climate change. In the studies to be carried out in this area, it is crucial to determine the drought-resistant origin and even individuals. For this purpose, the method used in the study can be recommended as a method that can be used to evaluate a large number of individuals in a short time, giving fast, cheap and reliable results.

Competing Interest / Conflict of Interest

The authors declare no conflict/competing interests.

Author Contribution

We declare that all authors equally contribute.

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Examination of Structural Characteristics and Biosecurity of Sheep Farms in Niğde Province

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ABSTRACT

The aim was to examine the structural features and biosecurity practices of sheep farms operating in Niğde province. For this purpose, 75 farms operating in the region were classified according to size (number of animals) and production system (extensive and intensive) and, they were compared in terms of typology and biosafety. In this context, a face-to-face survey was conducted with the owners or authorized persons of the small, medium and large size farms and the data collected from the farms about technical, sanitation-hygiene and health protection were comparatively presented. According to the findings obtained from the study, manure and wastes produced in 24% of farms were seen randomly throwing into the environment, and the differences observed between farms depending on the farm size were found significant ($P<0.05$). These farms can become a potential source of environmental and odor pollution. In addition, it was determined that disinfection was not applied to a large extent (97.30%) as a preventive measure at farm and shelter entrances ($P>0.05$). At the end of the study, it has been concluded that the typology and biosafety practices could be an important support for future strategic programs against disease and other factors which affects the production of the Niğde region.

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Introduction

The precautions taken to ensure and protect the continuity of animal health can be defined as “biosecurity”. It can be said that animal welfare for farms with successful biosecurity practices will be high and accordingly the yield will increase. However, biosecurity practices are often neglected in Türkiye, especially in sheep and goat production. Although the costs of taking biosecurity precautions may seem initially unnecessary, these practices will not only protect animals, but also support profitable production by increasing productivity. In addition, it is an issue that should be considered pollution that will occur during intensive use of natural resources in order to increase production per unit area. In this context, the issues of protecting natural life and organic production gain importance. At the same time, the negative effects of the uncontrolled and intensive use of some substances on biology cause the existence of some new diseases. Greenhouse gases emerging in animal production and the inability to effectively manage waste cause pollution of the environment. Depending on this, some unfavourable conditions may arise in production and human health. The role of small-scale producers in the livestock sector is not given much importance. However, in case of a health problem that may arise in the biosecurity management of small-scale farms, large-scale farms will also be at risk in terms of sustainability. For this reason, small-scale farms that have not taken biosecurity precautions are defined as high-risk groups in animal production When manure or waste resulting from animal production is not stored under appropriate conditions, it can become a potential source of pollution by creating environmental, visual and accompanying odour pollutions (Atılgan et al., 2006). Since the manure kept in unsuitable open conditions will cause environmental pollution, the manure must be stored in closed storage for future evaluation. For this reason, it is recommended to plan manure storage where the manure produced in the farms can be properly stored within certain periods (Karaman, 2006).

Since sheep production in Türkiye is predominantly small-scale and based on pasture, the animal products obtained constitute the main food source of agricultural farms, and accordingly, income from sheep production is generally at low levels. For this reason, efficient and profitable production should not be expected in every region of Türkiye with widespread production. However, sheep production should be made more profitable and sustainable in order to increase the income of sheep production farms from this production branch and thus their contribution to the Turkish economy. In this case, it is important to accelerate the concentration in regions where sheep production can become widespread and to ensure sustainable production in other regions. At this point, the most important question is which production system will be used where and how. It is very important to determine and define the structural and production characteristics of the existing sheep and goat farms in that region.

In the current study, the status of biosecurity practices and their structural characteristics in sheep farms operating at different scales and systems in districts of Niğde province were revealed. Also; there is almost no data on the level of biosecurity practice in livestock farms across Türkiye. Based on this, there has been no previous study to determine the biosecurity level of sheep farms in Niğde province. In particular, work-based studies by way of sampling in the field are of great importance in defining the problems in production and revealing their solutions. It has been concluded that such a study is necessary and important, taking into account the importance of ovine production activities from past to present, the presence of animals, their place in the country's agenda and their production capacity. In this study, it is aimed to raise awareness about the importance of biosecurity, the issue of biosecurity, especially in the province or the area where it is applied.

Material and Methods

In the study, face-to-face surveys with the owners or responsible persons of 75 sheep farms in the districts of the Niğde province in Türkiye were constituted to data of the study. The surface area of Niğde province is 7312 km² and its altitude is 1229 meters. It is also located between 34° 33′ 0″ East longitude and 37° 52′ 59″ North latitude. The survey areas are shown in Figure 1.

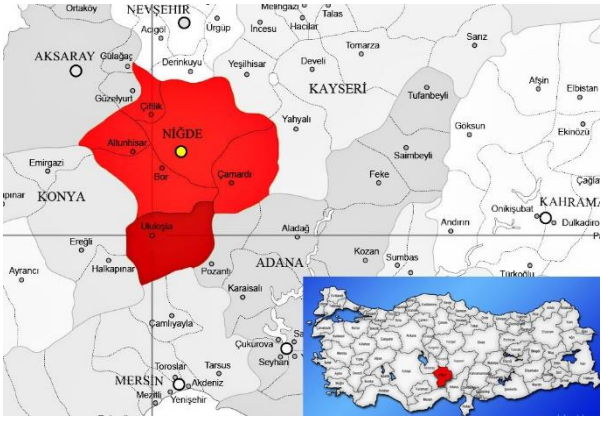

Figure 1. Districts of the Niğde province

A total of 75 surveys were conducted in Niğde/Center (2), Ulukışla (55), Çiftlik (1), Altunhisar (5), Bor (8) and Çamardı (4) districts. Farms were determined according to data obtained from the Niğde Directorate of Provincial Agriculture and Forestry. Subsequently, a survey was conducted that allowed analyzing these farms in terms of both typological and biosecurity points of view. The farms participating to the survey were selected to represent best the districts in terms of animal existence and production system. Accordingly, 25 farms were selected for each scale, including small (100 animals and less), medium (100-300 heads) and large (larger than 300 animals). Primarily the production type, housing, care and feeding methods of the farms with 24 questions prepared within the scope of the survey were determined and then the status of the farms on animal diseases, hygiene, manure and waste managements were revealed. Simple random sampling method was used to determine the number of farms to which the surveys were applied. The number of farms to represent the population in districts of Niğde province was found with the help of the following formula.

$$n = (N\ x\ p\ x\ q\ x\ 1.96^2)/((N-1)\ x\ d^2)$$

Since the number of farms in districts of Niğde province is N=539, if the sheep presence rate is assumed as p=0.5, the sample size necessary to estimate a 5% error level with q=1-p, d=0.12 deviation is approximately 67 farms. The demographic characteristics (Table 1) of the farms formed the study material were determined with nine questions asked in the survey.

Statistical analysis of the collected data was made with the help of the SPSS program. Technical, sanitation-hygiene and health protection analysis of farms depending on farm scales and production systems were done with the Chi-Square independence test.

Results and Discussion

The status of both typology and biosecurity of the farms determined were analysed by the survey. The findings of the research were examined as three sub-headings; technical, sanitation-hygiene and health protection as given below.

Technical Analysis of Farms

Türkiye with its natural and economic conditions, agricultural structure and traditions is a country suitable for sheep and goat production. In addition, considering Türkiye's geographical structure and pasture areas, it has been considered that sheep production is a low-cost extensive production activity. In the present study, 41% of the sheep farms in the districts of Niğde province were determined members of the national farmer registration system. The purpose of production in these farms was as follows; meet the family needs at 9.3%, provide financial income at 57.3% and as a habit at 32.0%, respectively. According to the present study, it has been revealed that the main purpose of farms making sheep production is to maintain a living by providing financial income. This finding was similar to the results of studies conducted by Karaman et al. (2012), Karakuş and Akyol (2013), Altınçekiç (2014) and Yerebakan (2017).

Table 1. Demographic characteristics of the farms

Parameters	Frequency	Percent (%)
The main purpose of sheep production		
Meeting family needs	7	9.30
Financial gain	43	57.30
Habit	24	32.00
All	1	1.30
National registration system membership		
Yes	41	54.70
No	34	45.30
The production system		
Intensive	10	13.30
Extensive	65	86.70
Type of barn		
No barn	4	5.30
Tarpaulin	17	22.70
Under of house	22	29.30
Reinforced concrete	31	41.30
Other	1	1.30
The number of employees in farm		
1-3	6	8.00
4-6	57	76.00
7 and more	12	16.00
The main source of the farm incomes		
Animal husbandry	70	93.30
Other	5	6.70
Dealing with non-animal production branches		
Yes	46	61.30
No	29	38.70
Farm owner's experience (years)		
0-9	6	7.90
10-19	16	21.30
20-29	31	41.20
30 and more	22	29.30
Farm scale		
Small (less than 100 heads)	25	33.33
Medium (between 100-300 heads)	25	33.33
Large (more than 300 heads)	25	33.33
Total	75	100

According to the small, medium and large scale status of the farms in the region where the study was conducted, the number of employees was determined between 4-6 people at a large rate such as 57.1%, 88.9% and 84.0%, respectively. Karakaya and Kızıloğlu (2014) reported that more than 4 employees in 75% of the farms that were small ruminant production in Bingöl province. In another study that examined the general structure of small ruminant production in Van province, the researchers reported that 9.1% of farms were operated by one person, 32.6% with two people, 28.8% with three people, 20.7% with four people and 8.8% with five people (Gezici, 2018). Although the number of employees in farms was diverse but in general operated by between 4-6 people. The most important factors affecting the number of employees of farms were the number of family individuals and the scale of the farm. In the current study, 38.70% of the farms were detected only engaged in sheep production, while 61.30% were engaged in other agricultural production together with sheep production. The rate of small, medium and large-scale farms engaged in different agricultural activities together with sheep production was almost 78.6%, 66.7% and 40%, respectively, this rate was observed to decrease while the scale rises up. In the studies conducted in Sivas, Muğla and Van provinces on the general structure of sheep production, the rate of farms engaged in non-livestock agriculture branches was determined %78,48, %48,0 and %96,0, respectively (Gezer, 2010; Aydın and Keskin, 2018; Gezici, 2018). In this context, the results of the current study were consistent with the literature, and it was revealed by the studies that sheep production was carried out together with various agricultural branches depending on the region and the scale of the farm. Due to the cold and snowy winter months in Niğde province, reinforced concrete barns were seen used the most by 41.30%, followed by under of houses with 29.30% and tarpaulin barns with 22.70%. The findings of the current study were similar to the results of the studies conducted by Karaman et al. (2012) and Meşe (2019) on the type of barns. Although the barns were seen generally cheap and primitive in sheep production in Türkiye, the characteristics, availability and cost of the material, especially the climatic conditions affect the structure of the barn. It was found that 70.50% of the surveyed farm

owners had a work experience of 20 years and more, and sheep production in the region was the main source of income for 93.30% of breeders. These results show that sheep production was carried out in the region for many years and it was an important production area for the region. It was detected that production was continued by breeders with at least 10 years of experience in many studies where the experience periods of breeders engaged in sheep production are examined (Acar and Ayhan 2012; Karakaya and Kızıloğlu 2014; Türkan, 2017; Aydın and Keskin, 2018; Karadaş, 2018). The declarations of the breeders engaged in sheep production for many years were consistent with the results of the present study.

The technical criteria (Table 2) of farms were detected with eight questions asked in the survey.

According to this, all the animals in region grazed on pasture almost 10 months of the year. This finding was similar to many studies that the farms operating sheep production in different regions of Türkiye benefit from pasture to a large extent (Gezici, 2018; Karagöz, 2019). Although the nutrition of small ruminant animals in Türkiye is largely dependent on pasture, there are many studies reporting that the available pastures are insufficient in terms of quantity and quality (Aksoy and Yavuz, 2012; Yerebakan, 2017; Bakır and Mikail, 2019). The production objectives of farms in sheep production may vary depending on the habits of the producer and market opportunities (Koyuncu et al. 2006). The primary production of the sheep farms in the region was determined meat production. The differences observed in milk, wool and manure production depending on the scale of the farms were found significant (P<0.01), while the differences observed in milk and wool production depending on the production system were significant (P<0.01).

Table 2. Technical analysis of the farms

Parameters	Frequency (%)		P	
			SC	PS
Grazing state in pasture				
Yes	75 (100.00)		-	-
No	--			
Production direction	Yes	No		
Meat	74 (98.70)	1 (1.30)	0.329	0.133
Milk	20 (26.70)	55 (73.30)	0.002**	0.001**
Wool	23 (30.70)	52 (69.30)	0.001**	0.001**
Manure	42 (56.00)	33 (44.00)	0.001**	0.170
The feeds used in feeding	Yes	No		
Barley	43 (57.30)	32 (42.70)	0.069	0.174
Corn	22 (29.30)	53 (70.70)	0.001**	0.055
Bran	14 (18.70)	61 (81.30)	0.027*	0.384
Hay	73 (97.30)	2 (2.60)	0.438	0.250
Alfalfa	45 (60.00)	30 (40.00)	0.135	0.005**
Silage	35 (46.70)	40 (53.30)	0.361	0.321
Pulp	26 (34.70)	49 (65.30)	0.023	0.002**
Concentrate feed	68 (90.70)	7 (9.30)	0.859	0.005**
Other	16 (21.30)	59 (78.70)	0.108	0.999
Storage state of feed				
Feed warehouse	40 (53.30)		0.002**	0.451
Under of house	11 (14.70)			
Under of tarpaulin	14 (18.70)			
Other	10 (13.30)			
Availability of different animals in feed warehouse and barn areas				
Yes	18 (24.00)		0.803	0.695
No	57 (76.00)			
The use state of manure	Yes	No		
I'm selling	20 (26.70)	55 (73.30)	0.168	0.452
I'm throwing	18 (24.00)	57 (76.00)	0.031*	0.999
I'm using myself	39 (52.00)	36 (48.00)	0.007**	0.999
The distance between manure store and barn				
0-20 m	48 (64.00)		0.035	0.275
21-30 m	5 (6.60)			
31 m more	18 (24.00)			
I can't store	4 (5.30)			
Availability of different animals in manure store				
Yes	34 (45.30)		0.686	0.497
No	41 (54.70)			

SC-Scale; PS-Production system. **Significant at the 1% probability level, *Significant at the 5% probability level.

The number of farms producing milk, wool and manure in addition to meat production in small-scale farms was observed to be higher than the other sizes, and the number of farms producing these products decreased while the size increased. In addition, in terms of production type, milk and wool production was found significantly higher in extensive system than intensive system. Keskin (1996) reported similar findings to these results. The researcher reported that sheep production was carried out primarily for the purpose of meat production. Some farmers have not milked their ewes and left the whole milk to the newborns up to 4-5 months. Hay, concentrated feed and barley were determined used extensively in the nutrition of sheep in the region (Table 2). There were significant differences in using corn ($P<0.01$) and bran ($P<0.05$) depending on the scale of the farms. The significant differences between farms in terms of alfalfa, pulp and concentrated feed used in the feeding of animals were observed depending on the production system ($P<0.01$). The use of corn and bran in small-scale farms was significantly higher than in medium and large-scale farms, and corn was not detected used in the feeding of animals in large-scale farms. While alfalfa was used in all farms in the intensive production system, it was observed that the rate of use of alfalfa in the extensive system was 53.80%. Also, the use of pulp for feeding the animals in the intensive system was significantly higher than in the extensive system. Although the feed raw materials used in sheep feeding vary according to the scales and production systems of farms, sources such as barley, wheat, corn, pulp, bran, alfalfa, corn silage and concentrated feed were mostly found used on farms (Karakuş and Akyol, 2013; Yerabakan, 2017; Aydın and Keskin, 2018). The storage status of feeds varied depending on the scale of the farms ($P<0.01$). It was determined that 53.30% of the farms were stored in the feed warehouse. Small and medium-sized farms kept their feedstuff mostly in the feed warehouse, while the feeds on large-scale farms were kept equally in the warehouse, under the houses, under the tarpaulin and other options.

The uses of manure produced in the farms were seen to vary depending on the scale of the farms and their production in other agricultural branches. The manure obtained in small-scale farms was mostly used to meet their own needs such as their field and/or their vegetable production. In addition, the manure obtained from big scale farms was sold or discarded. Özsayın and Everest (2019) reported that 92.8% of the producers engaged in sheep production in Çanakkale Gökçeada used their manure in own agriculture production. In similar studies, the researchers reported that was revealed that sheep breeders in Ardahan, Karaman and Yozgat provinces used own land of a significant part of manure obtained from animal production and the remaining part was sold or was evaluated as fuel (Şahinli 2014, Demir et al. 2015; Tamer and Sariozkan, 2017). In a study conducted in Van province, it was stated that 44.6% of the breeders used their manure as fuel in winter period (Karakuş and Akkol, 2013).

Sanitation and Hygiene Analysis of Farms

The cleanliness and hygiene practices in animal production may affect the welfare of animals and cause positive or negative changes in the amount of yield obtained from them. In addition, in cases where cleaning and hygiene conditions are not provided at enterprises, different diseases may occur in animals depending on poor environmental conditions. In this context, it is known that basic cleaning and hygiene practices applied in enterprises are an important part of biosecurity. The sanitation and hygiene criteria (Table 3) of the farms were revealed with eight questions asked in the survey. The barns were seen usually cleaned once a month on the farms where the study was carried out, and significant differences were observed in the cleaning times of the barn, feeder and waterer depending on the scale and production system of the farms (Table 3). As the scale of the farm grows, barn cleaning ($P<0.05$) was observed doing more often, while the feeder and waterer cleaning ($P<0.01$) was seen less frequently. In addition, when the farms were compared according to the production systems, the cleaning of barn, feeder and waterer was detected more frequently in intensive production ($P<0.01$). In a study conducted by Altınçekiç (2014), barn cleaning was determined usually done once a year in sheep farms operating in Bursa province. Although similar results were found in the study conducted by Kılıç et al. (2013) and Alkan et al. (2013) state that barn cleaning was mostly done daily. The differences observed in the cleaning frequency of barn, feeder and waterers; it was thought to be caused by parameters such as animal presence, ground type of barn, climate and number of employees and evaluation of manure. In the farms where the study was conducted, a large part such as 93.30% of the breeders reported that they had special work suits for the farm. Contrary to the results of the study, Altınçekiç (2014), who examined the structural status of sheep farms in Bursa province found that none of the breeders wore special work clothes during the feeding or milking of sheep in farms, and breeders thought that such an application was unnecessary. In addition, the precaution taken during the treatment process of sick animals in the intensive production system were determined to be more stringent ($P<0.05$). Regardless of the scale and production type of the farms; it was stated that barns were ventilated during the winter months, disinfection was applied at farm and barn entrances, and waste such as injector were thrown to trash after vaccine and drug application ($P>0.05$). It was determined that the measures taken during the treatment of sick animals were significantly affected by the production system of the farms ($P<0.05$). Although protective measures are taken during the treatment of sick animals in all farms with intensive production systems, it has been reported that these measures are not taken into account in a large proportion of 32.3% in farms with intensive production models.

Health Protection Analysis of Farms

The presence of diseases in livestock farms can cause economic losses. Diseases occurring in animals lead to economic losses in the farms as a result of death and yield losses, high treatment costs, and time and effort losses. For this reason, it can be said that biosecurity precautions applied in animal production will support profitable production in terms of farms' economy. Regardless of the scale and production system, 98.70% of the breeders were seen to receive veterinary support in case of any disease in the farms. In addition, it was stated that the treatments and vaccines were applied by themselves in some of the small-scale farms. In a similar study conducted on sheep farms in Bursa province, it was stated that vaccination practices of

breeders mainly had performed by veterinarians, and some breeders performed by veterinary health technicians or themselves of these practices (Altınçekiç, 2014). In studies conducted on farms operating sheep production in various regions, veterinary service was stated received at a high rate in case of any disease or application of vaccination (Karakuş and Akyol, 2013; Yerabakan, 2017; Özsayın and Everest, 2019). In the region conducted the present study was seen that the biggest health problem of the farms was lameness.

The most frequently applied vaccines during the year were determined Antivariolic, Brucella, Plague and Enterotoxaemia vaccination (Table 4). Internal-external parasites (P<0.05) and lameness (P<0.01) among the diseases observed on farms, and in terms of Enterotoxaemia (P<0.01) in the applied vaccines were found significant differences observed depending on the scale of farms. While internal-external parasites were observed intensely in small-scale farms, this problem was determined to decrease as the scale grows. However, the lameness in the farms was observed to increase with the growth of the scale. In addition, the Enterotoxaemia vaccine was determined mostly applied in small-scale farms and the application of this vaccine was observed to decrease as the scale grows. As well as ticks and fleas within external parasites depending on farms's size were a common problem, it was determined that 81.30% of the farms had a housefly problem. The production system was determined a significant effect on the housefly problem observed in the farms and the housefly problem was more common in the extensive system (P<0.05). In addition, the differences observed depending on the scale of farms on tick and lice observed as external parasites were detected to be significant (P<0.01). Lice were seen more intensively on small-scale farms, and this problem was seen to decrease with the growth of the farm scale. Generally, no scientific techniques for health protection are used in extensive farms, especially in the fighting against external parasites. Instead, some techniques traditionally applied by breeders are used. These techniques; It is carried out by searing, spraying with some herbal substances, treatment with diesel fuel or similar methods.

Table 3. Sanitation and hygiene analysis of the farms

Parameters	Frequency (%)	P	
		SC	PS
Cleaning state of barns (times)			
Every day	1 (1.30)	0.040*	0.001**
Once a week	9 (12.00)		
Once a month	48 (64.00)		
Once every three months	7 (9.30)		
Once every six months	8 (10.70)		
Not cleaning	2 (2.70)		
Cleaning state of feeders (days)			
1-3	19 (25.30)	0.008**	0.001**
4-7	20 (26.70)		
8 and more	35 (46.70)		
Not cleaning	1 (1.30)		
Cleaning state of waterers (days)			
1-3	21 (28.00)	0.001**	0.001**
4-7	30 (40.00)		
8 and more	22 (29.30)		
Not cleaning	2 (2.70)		
Ventilation state in winter of the barns			
Yes	72 (96.00)	0.431	0.645
No	3 (4.00)		
Disinfection application at the entrance of the farm and barn			
Yes	2 (2.70)	0.500	0.343
No	73 (97.30)		
The use of specific work suits for farm			
Yes	70 (93.30)	0.450	0.999
No	5 (6.70)		
Preventive measures in the treatment of sick animals			
I'm wearing gloves	41 (54.70)	0.179	0.013*
I'm wearing a mask	-		
I wear gloves-mask	13 (17.30)		
Other	-		
I don't take precautions	21 (28.00)		
The state of the waste like injector after the application of the vaccine or drug			
I throw to trash	73 (97.30)	0.342	0.748
I throw randomly	1 (1.30)		
I'm reusing	1 (1.30)		

SC-Scale; PS-Production system. **Significant at the 1% probability level, *Significant at the 5% probability level.

Table 4. Health protection analysis of the farms

Parameters	Frequency (%)		P	
			SC	PS
In case of illness of animals	Yes	No		
I treat myself	8 (10.70)	67 (89.30)	0.168	0.999
I get support from other farmers	4 (5.30)	71 (94.70)	0.108	0.999
I get support from the pharmacist	3 (4.00)	72 (96.00)	0.187	0.999
I get support from veterinarians	74 (98.70)	1 (1.30)	0.329	0.999
Common disease in farm	Yes	No		
Internal-external parazite	10 (13.30)	65 (86.70)	0.031*	0.124
Lameness	61 (81.30)	14 (18.70)	0.003**	0.999
Enterotoxemia	22 (29.30)	53 (70.70)	0.433	0.467
Brucellozis	14 (18.70)	61 (81.30)	0.527	0.677
Other	9 (12.00)	66 (88.00)	0.194	0.999
The vaccines applying to animals	Yes	No		
Brucella	71 (94.70)	4 (5.30)	0.187	0.999
Plague	49 (65.30)	26 (34.70)	0.790	0.478
Pox	73 (97.30)	2 (2.70)	0.105	0.999
Lameness	12 (16.00)	63 (84.00)	0.741	0.999
Enterotoxemia	34 (45.30)	41 (54.70)	0.001**	0.497
Alum	11 (14.70)	64 (85.30)	0.664	0.001
Internal-external parasites	4 (5.30)	71 (94.70)	0.106	0.443
External parasites observed in animals	Yes	No		
Tick	35 (46.70)	40 (53.30)	0.001**	0.500
Lice	8 (10.70)	67 (89.30)	0.002**	0.068
Flea	45 (60.00)	30 (40.00)	0.135	0.298
Not external parasite	23 (30.70)	52 (69.30)	0.003**	0.714
Housefly problem in farm				
Yes	61 (81.30)		0.527	0.016*
No	14 (18.70)			
Isolation application at new animal entry into the herd				
Isolation does not apply	47 (62.70)		0.137	0.154
Isolation is applied for 7 days	18 (24.00)			
Isolation is applied for 14 days	7 (9.30)			
Isolation is applied for more than 14 days	3 (4.00)			
The grazing of animals in pastures where sick animals are found				
Yes	68 (90.70)		0.005**	0.046*
No	7 (9.30)			
The state of dead animals				
I throw randomly	6 (8.00)		0.155	0.030*
I'm burying	29 (38.70)			
I'm burying with lime	16 (21.30)			
I throw in the water	24 (32.00)			

SC-Scale; PS-Production system. **Significant at the 1% probability level, *Significant at the 5% probability level.

A study examining the structural status of sheep farms in Antalya province found that 95.6% of farms had performed Enterotoxaemia, 85.6% Alum, 84.4% Brucella, 76.7% Blue Tongue, 67.8% Plague and 63.3% Smallpox vaccines during the year (Yerebakan, 2017). However, the incidence of the housefly problem, which was caused great economic damage to the farms was reported as 92.3% by researchers. In a similar study conducted by Karakuş and Akyol (2013) in Van province, only 46.19% of all farms reported that they had performed Enterotoxaemia, Smallpox, Brucella and Alum vaccines in animals. In the same study, the most common health problems in farms were reported external parasites with 65.36% and respiratory tract diseases by 52.19%. In another study conducted in Bursa province, the main vaccines commonly used against epidemic diseases observed in farms was found to be Smallpox, Alum, Brucella, Enterotoxaemia and Anthrax (Altınçekiç, 2014). All these results were consistent with the findings of the current study, and the observed disease types and the vaccines applied in sheep production operating in different regions of Türkiye were revealed largely similar.

Quarantine was determined to not applied to the animals that were added to the herd from other farms in the majority of the farms regardless of the scale or production system. In a study conducted in Bursa province contrary to the current study, all breeders were reported to apply quarantine to animals at new animal entrances to the farm (Altınçekiç, 2014). The breeders in the present study stated that they grazed their animals in the pastures where the sick animals were found and they did not take any precaution in this situation. In this context, the differences between farms in terms of farm scale ($P<0.01$) and production system ($P<0.05$) were determined to be significant. The grazing

status of animals in pastures existing of diseased animals was determined to be the lowest in small-scale farms and the highest in large-scale farms. Although the practices on the condition of the dead animals in the farms differ, the dead animals were determined to be buried at a high rate in farms with an intensive production system, and they were thrown into the water or buried in extensive farms ($P<0.05$).

Conclusion

At the end of this study, it has been observed that biosecurity precautions were not taken, sanitation and hygiene rules were not followed on the farms in Nigde province, as a result of these, the breeders complain about animal health. It is thought that the most effective solution to eliminating these complaints and deficiencies can be achieved through correct planning, training, awareness-raising and incentives activities. Thus, it has been concluded that the typology could be an important support for the future strategic programs against disease and hygiene, which to date affect the production of our region.

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Conflict of Interest

No potential conflict of interest relevant to this study was reported.

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Evaluation of Quality Characteristics of Commercial Fermented Sausages (Sucuk and Heat-Treated Sucuk)

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ABSTRACT

The study aimed to evaluate the pH, water activity (a_w), residual nitrite, lactic acid bacteria, *Micrococcus/Staphylococcus*, Enterobacteriaceae and yeast-mould in fermented sausage samples from different firms. A total of 30 sucuk and 30 heat-treated sucuk samples were taken from 10 different brands with different batch numbers. According to analysis results, all samples, with the exception of some heat-treated sucuk samples from one brand, provided pH values that were within the permitted limit of regulation. For sucuk, mean a_w value of only one brand was below 0.90, while a_w values for heat-treated sucuk were in the range of 0.928 to 0.957. All samples had residual nitrite levels less than 15 mg/kg (in the range of 7.84-14.80 mg/kg). Yeast-mould and Enterobacteriaceae numbers were often below <2 log cfu/g. The number of *Staphylococcus and Micrococcus* showed a wide variation in both products which was <2 - 5.96 log cfu/g for sucuk and <2 - 7.85 log cfu/g for heat-treated sucuk. Lactic acid bacteria counts varied between 2-<4.0 log cfu/g in 40% of heat-treated sucuk samples. In sucuk, the number of lactic acid bacteria was <6 log cfu/g in 23.33% of the samples, and 6-<8 log cfu/g in 50% of the samples.

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Introduction

Fermented sausages that maintain popular with their typical flavors and aromas may differ depending on raw material quality, added ingredients, and process conditions (Yu et al., 2021). Fermented sausages are classified by water activity (a_w), weight loss, moisture, moisture:protein ratio, geographical region, degree of fat comminution. They are categorized into three groups based on their moisture content: high humidity (50–60% water), semi-dry (35–50%), and dry (20–35%) sausages (Campbell-Plantt, 1995). Fermented sausages involve dry or semi-dry products such as chorizo, hard salami, peperroni, salami, summer sausage, Rohwurst, cervelat (Kaya and Kaban, 2020). However, in many countries, especially in traditional products, there isn't broad, explicit and uniform distinction among semi-dry and dry fermented sausages, and even many products with the same name can be manufactured as "dry" or "semi-dry" fermented sausages (Lücke, 2107). On the other hand, high humidity fermented sausages such as Mettwurst and Teewurst, as well as Sobrasada made in several countries, are soft-consistent products made with fermentation (Kaya and Kaban, 2020).

Nitrite, pH, a_w and low oxygen levels are important hurdle effects to microbial stability in fermented sausages. In fermented sausages, salt (2-3%) exhibits partial bacteriostatic activity and lowers the water activity of the batter to 0.955-0.960, depending on the fat ratio in the formulation (Kaya and Kaban, 2020). a_w of dry fermented sausages is less than 0.90, and no smoking or heat treatment is used in the manufacturing process. a_w in semi-dry fermented sausages ranges between 0.90-0.95, and a heat treatment of 60-68ºC is typically used (Caplice and Fitzgerald, 1999). The rate and degree of acid production during fermentation in fermented sausages are critical for product sensory characteristics and product safety. For controlled acid formation in industrial manufacturing, starter culture has been involved, as opposed to the spontaneous flora or back slopping application that occurs in traditional production (Kaban et al., 2022a). Semi-dry fermented sausages differ substantially from dry fermented sausages in that they have a strong distinctive flavor due to the rapid fermentation. The final pH of these products ranges from 4.7 to 5.4. However, the pH varies between 5.2 and 5.8 in dry fermented sausages, which ripened longer

than semi-dry fermented sausages (Vignolo et al., 2010). According to the Turkish Food Codex Communiqué on meat, prepared meat mixtures and meat products, the maximum pH value authorized for sucuk is 5.4, while the maximum pH value allowed for heat-treated sucuk is 5.6 (Anonymous, 2019). In fermented sausages, nitrite and/or nitrate are utilized as curing agents depending on the product type and processing conditions. The fermentation process is the most important part in the production, and the level of nitrite acts as a hurdle effect for many pathogenic bacteria. Ingoing nitrite level permitted in these products is limited to 150 mg/kg. Low residual nitrite levels in the final product are also crucial for reducing the nitrosamine risk (Sallan et al., 2023).

Lactic acid bacteria and Gram (+) catalase (+) cocci are technologically essential microorganisms for fermented sausages (Kaban et al., 2022a). Lactic acid bacteria, which multiply up to around 10^8 cfu/g in the fermentation stage, retain their survival to a great extent in the further stages of ripening (Dalmış and Soyer, 2008; Soyer et al., 2005). The number of Gram (+) catalase (+) cocci in these products varies depending on the acidification during fermentation (Akköse et al., 2023). These microorganisms, which are important in the microbiota of fermented sausages, are found at lower levels in heat-treated sucuk. On the other hand, the growth of Gram (-) flora such as Enterobacteriaceae and *Pseudomonas* is suppressed by hurdles such as nitrite, pH, redox potential, a_w and their combinations (Kaya and Kaban, 2020).

Two different types of fermented sausage are manufactured in Türkiye, namely sucuk and heat-treated sucuk. While fermentation and drying procedures are used in sucuk production, fermentation, heat application, and drying procedures are applied in the production of heat-treated sucuk (Armutçu et al., 2020). The moisture:protein ratio and pH value of sucuk are the most crucial product attributes. The moisture:protein ratio should be less than 2.5, and the pH should be no more than 5.4 in sucuk. Heat-treated sucuk is permitted to have a greater moisture/protein ratio (3.6) and a higher pH (up to 5.6) value (Anonymous, 2019). Many studies have been carried out on the quality attributes of sausage samples obtained from market (Yücel and Karaca, 1993; Sancak et al., 1996; Atasever et al., 1998; Çon et al., 2002; Kaban and Kaya, 2008; Sezer et al., 2013; Büyükünal et al., 2016; Kızılkaya et al., 2023). On the other hand, there are little information on quality attributes of heat-treated sucuk obtained from the market (Sezer et al., 2013; Kaban et al., 2022b). No research investigating the variations in quality traits between the same brand's sucuk and heat-treated sucuk based on different production durations has been also found in the literature.

The aim of the study is to determine and analyze pH and water activity values as well as residual nitrite content of sucuk and heat-treated sucuk samples received from various firms at different times. In addition, it is aimed to determine the numbers of technologically important microorganisms (Lactic acid bacteria and *Micrococcus/Staphylococcus*), Enterobacteriaceae and yeast-moulds in these products.

Material and Method

Material

Samples of sucuk and heat-treated sucuk that were collected from ten different brands in Türkiye were utilized as study materials. Vacuum packed samples were gathered from retail establishments while considering different batch numbers at three different times. The following analyses were performed on a total of 60 samples (10 sucuk and 10 heat-treated sucuk samples) which are 3 samples from each brand.

Method

Physicochemical Analysis

Water Activity (a_w): The equipment (TH500 a_w Sprint, Novasina) was utilized to determine the a_w value of the samples. The instrument was calibrated using six different salt solutions prior to use. At 25ºC, water activity was measured (Kaban et al., 2022a).

pH: 10 g samples were weighed into jars and homogenized with distilled water (100 mL) with ultra turrax (IKA Werk T25, Germany) to detect pH value. The pH meter (Mettler Toledo, Greifensee, Switzerland) was calibrated using buffer solutions of pH 4.0 and 7.0 before analysis (Kaban et al., 2022a).

Residual Nitrite: The samples homogenized were weighed at 10 g with a 0.1 mg reliability. Ultrapure water (50 mL, 50-60°C) was added and then well mixed using glass baguette. The mixture was then transferred to flasks of 200 mL capacity. Acetonitrile (50 mL) was added to the flask following 15 minutes of mixing, after which 200 mL of ultrapure water was added. The resulting samples were filtered using nitrite-free/nitrate-free filter paper, and the filtrates were then put in vials after being run through a 0.45 µm filter. Using HPLC/DAD, the residual nitrite concentration was calculated. The flow rate in the column was set to be 2 mL/min and the nitrite standard was used for identification. The results were obtained in mg/kg (NKML, 2000).

Microbiological Analysis

Sausage sample of 25 g was homogenized with 225 mL of sterile physiological saline in Stochmacher (Lab Stomacher Blander 400 - BA 7021). From this homogenate, serial dilutions were prepared. The lactic acid bacteria, *Micrococcus/Staphylococcus*, Enterobacteriaceae, and yeast-mould counts of the samples were determined using the spread plate technique. The results are given as log cfu/g.

Lactic Acid Bacteria: The number of lactic acid bacteria was determined using MRS Agar (de Man Rogosa Sharpe Agar, Merck). The petri plates were incubated in anaerobic conditions (Anaerocoult A, Merck) for 2 days at 30°C after adding 0.1 mL of each dilution to the medium. Catalase (-) colonies were counted at the end of the incubation to determine the number of lactic acid bacteria (Yılmaz Oral and Kaban, 2021).

Micrococcus/Staphylococcus : MSA (Mannitol Salt Phenol Red Agar, Merck) was used for the number of *Micrococcus/Staphylococcus* and the inoculated plates were incubated at 30°C for two days. Following the incubation, the number was detected by considering the catalase (+) cocci (Yılmaz Oral and Kaban, 2021).

Enterobacteriaceae: In order to determine the Enterobacteriaceae number of the samples, 0.1 mL of the dilutions prepared were transferred to VRBD (Violet Red Bile Dextrose, Merck) agar plates and incubation was conducted at 30°C for 2 days under anaerobic conditions (Anaerocoult A, Merck). After incubation, the number of Enterobacteriaceae was detected by counting red, rose red or purple colonies larger than 1 mm (Gökalp et al., 2015).

Yeast and Mould: To count the number of yeast and mould, RBC (Rose Bengal Chloroamphenicol, Merck) was utilized. The dilution solutions were added to the petri plates, where they underwent a 5-day incubation period at 25°C. The colonies were counted after 5 days (Gökalp et al., 2015).

Statistical Analysis

In the study, fermented sausage type (sucuk and heat-treated sucuk) and brand (A, B, C, D, E, F, G, H, J, K) were taken as factors and the trials were based on randomized complete block design in 2x10 factorial design with 3 replications. Sampling was carried out at three different times with different batch numbers, and thus, a total of 60 samples, 30 of which were heat-treated sucuk and 30 sucuk samples, were examined. Analysis of variance was applied to pH, a_w and residual nitrite results in the study and the means of the results were compared with Duncan's multiple range test (Version 24, SPSS Inc., Chicago, IL, USA). For lactic acid bacteria and *Micrococcus/ Staphylococcus* numbers, graphs were prepared and evaluated using frequency distribution.

Results and Discussion

Physicochemical Results

The effects of fermented sausage type and brand factors on pH and a_w values are given in Table 1. According to the results, both fermented sausage type and brand factor showed a significant effect on pH and a_w values ($P<0.05$). The lowest mean values in terms of a_w and pH was found in sucuk. The mean pH value of both sucuk and heat-treated sucuk is under the limit values given in the Turkish Food Codex Communiqué on meat, prepared meat mixtures and meat products (maximum pH: 5.4 for sucuk; maximum pH: 5.6 for heat-treated sucuk) (Anonymous, 2019). There were also differences between the brands in terms of pH value. The brand × fermented sausage type interaction was given in Figure 1a.As can be seen from Figure 1a, pH values showed a wide variation in both types of fermented sausage samples. However, only pH values of sucuk and heat-treated sucuk samples from the B and E brands differed statistically. The mean pH in heat-treated sucuk sample of B brand (pH=6.1) was higher than the communiqué's limit value. In other brands, the pH value is below the specified limit values (Figure 1a) (Anonymous, 2019). Previous investigations on sucuk yielded similar findings (Yücel and Karaca, 1993; Kaban and Kaya, 2008). Additionly, Sancak et al. (1996) reported that the pH value for sucuk ranged between 4.99-6.21 (mean pH: 5.50), whereas Atasever et al. (1998) stated that the pH value for sucuk ranged between 4.45-6.43 (mean pH: 5.24). Mean pH of sucuk was found by Kızılkaya et al. (2023) to be below 5.4. However, in another research, the mean pH of sucuk was 6.18 (min: 4.94, max: 6.97) while heat-treated sucuk was 6.74 (min: 6.4, max: 6.92) (Sezer et al., 2013). Vural (1998) detected a pH range of 5.16 to 5.55 for semi-dry fermented sausage. Kaban et al. (2022b) also reported that pH value of commercial heat-treated sucuk samples varied between 4.28-5.47. High pH in sucuk and heat-treated sucuk indicates that either fermentation is not performed sufficiently or not performed at all. The pH value is an important hurdle for fermented sausages such as sucuk and heat-treated sucuk (Leistner and Gorris, 1995; Kaya and Kaban, 2020). In the present study, only the heat-treated sucuk of B brand did not comply with the regulation (Figure 1a) (Anonymous, 2019).

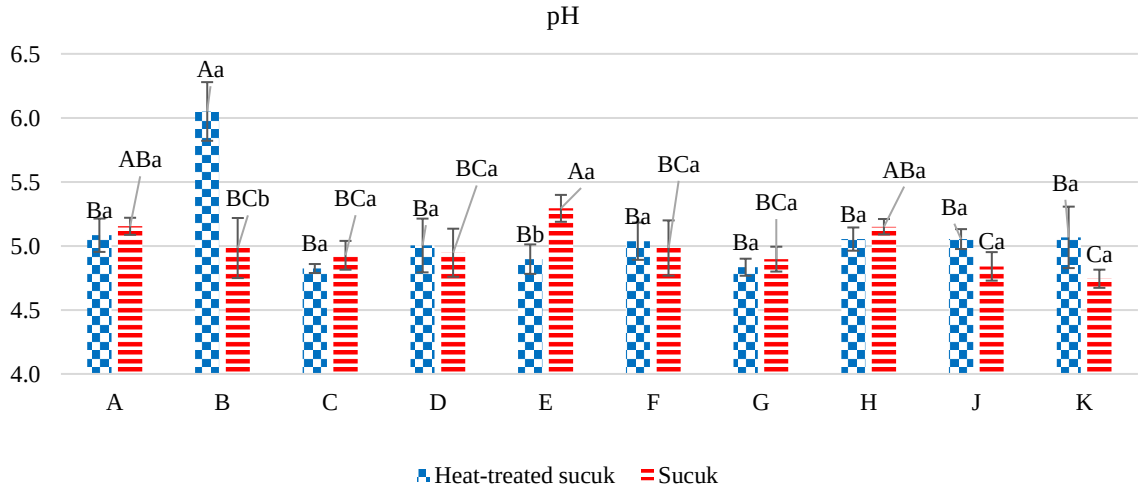
The brand × fermented sausage type interaction, which was found to have a very significant effect on the a_w value of the samples, is given in Figure 1b. a_w of sucuk and heat-treated sucuk was in the range of 0.880-0.950 and 0.928-0.957, respectively. As can be seen from Figure 1b, in brands of D, G and K, sucuk gave lower a_w value than heat-treated sucuk. On the other hand, there was no statistically significant difference in a_w among the samples of sucuk and heat-treated sucuk ($P>0.05$) (Figure 1b). As can be understood from the results, the a_w values of 0.94 - 0.95 indicate a limited degree of drying in these products. The a_w of dry fermented sausages is below 0.90, whereas a_w of semi-dry fermented sausages ranged from 0.90 to 0.95 (Caplice and Fitzgerald, 1999). In the present study, the mean a_w of sucuk was found below 0.90 only in D brand (mean a_w:0.88). In K brand, the mean a_w of sucuk was 0.905 (Figure 1b). Kızılkaya et al. (2023) found a_w values in the range of 0.937-0.961, while Kaban and Kaya (2008) in the range of 0.801-0.913 in the sucuk samples obtained from the market. As can be seen from Figure 1b, the mean a_w of heat-treated sucuk was quite high in D brand (a_w:0.957) and G brand (a_w:0.951). Kaban et al. (2022b) reported lower a_w values (min a_w: 0.913, max a_w: 0.940 mg/kg) for heat-treated sucuk. pH and a_w are considered as two significant hurdles in fermented sausages. The pH level of semi-dry fermented sausages with a higher a_w than dry sausages is generally below 5.0 (Sallan and Kaya, 2021). In the present study, while the a_w is high in the majority of the brands, the pH value is below 5.0. According to the a_w and pH results of this study, both sucuk and heat treated sucuk should be kept in cold storage.

In the study, there was no significant effect of fermented sausage type and brands factors on residual nitrite ($P>0.05$). Similarly, there was no significant effect of brand × fermented sausage type interaction on residual nitrite ($P>0.05$) (Table 1). Residual nitrite for both sucuk and heat-treated sucuk was below 15 mg/kg (Table 1). In a previous study on sucuk, higher residual nitrite amounts (min: 29.66 mg/kg, max: 89.0 mg/kg, mean: 46.87 mg/kg) were determined (Yücel and Karaca, 1993). Büyükünal et al. (2016) determined the mean nitrite level for sucuk as 24.83 mg/kg. In addition, Kızılkaya et al. (2023) determined the residual nitrite content in sucuk samples to be 5.81–17.65 mg/kg, and Kaban et al. (2022b) found the residual nitrite content in all heat-treated sucuk samples to be below 10 mg/kg. Low residual nitrite level is an important factor in terms of nitrosamine in fermented sausages. It is even more important to have low residual nitrite levels in products cooked before consumption such as sucuk and heat-treated sucuk (Sallan et al., 2023). Residual nitrite in fermented sausages, however, is vital for color stabilization, and a residual nitrite in the level of 10-15 mg/kg is widely suggested as a reservoir for the regeneration of cured meat color (Alahakoon et al., 2015).

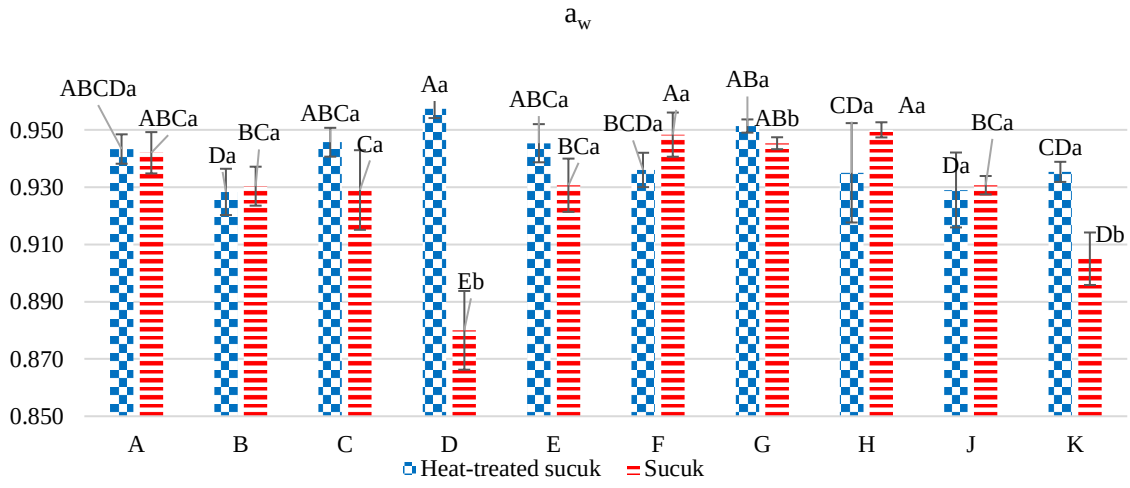
Table 1. Overall effect of fermented sausage type and different brands on pH, a_w and residual nitrite values in samples

Treatments	n	pH	a_w	Residual Nitrite (mg/kg)
Fermented Sausage Type (ST)				
Heat-treated sucuk	30	5.090^{a}	0.941^{a}	10.671^{a}
Sucuk	30	4.992^{b}	0.929^{b}	11.053^{a}
SEM		0.021	0.001	0.538
Significance		**	**	ns
Brand (Br)				
A	6	5.118^{b}	0.943^{ab}	9.602^{a}
B	6	5.517^{a}	0.929^{cd}	9.117^{a}
C	6	4.875^{c}	0.937^{bc}	10.868^{a}
D	6	4.975^{abc}	0.919^{e}	10.778^{a}
E	6	5.095^{ab}	0.938^{abc}	11.292^{a}
F	6	5.010^{abc}	0.942^{ab}	11.855^{a}
G	6	4.865^{c}	0.948^{a}	11.343^{a}
H	6	5.102^{ab}	0.943^{ab}	11.127^{a}
J	6	4.947^{bc}	0.930^{cd}	14.800^{a}
K	6	4.905^{c}	0.920^{de}	7.837^{a}
SEM		0.048	0.003	1.202
Significance		**	**	ns
ST×Br		**	**	ns

ns: not significant; **: P<0.01



(a)



(b)

Figure 1. Mean pH (a) and a_w (b) values of heat treated sucuk and sucuk samples obtained from different brands
(A-D: Different capital letters indicate significant differences between brands for fermented sausage type. a-b: Different lower case letters indicate significant differences between fermented sausage types for brand)

Microbiological Results

Lactic acid bacteria, *Micrococcus/Staphylococcus*, yeast-mould and Enterobacteriaceae counts of the brands with respect to the fermented sausage type are given in Table 2. Figures 2a and 2b, respectively, show frequency distribution graphs of lactic acid bacteria and *Micrococcus/Staphylococcus* counts of the samples. As can be shown from Table 2, lactic acid bacteria count in sucuk and heat-treated sucuk showed a wide variation. In addition, significant variations were also found between different batch numbers of the same brand (Table 2). For example, the lowest number of lactic acid bacteria was 3.80 log cfu/g and the highest count was obtained as 7.88 log cfu/g for sucuk from A brand. A similar situation exists in sucuk samples of B brand (min:2.48 log cfu/g, max: 7.90 log cfu/g). This circumstance was interpreted as an evidence that these firms do not produce in a standardized manner. As can be seen in Figure 1a, which was created by considering all sucuk and heat-treated sucuk samples, the number of lactic acid bacteria were found in 50% of the 30 sucuk samples at the level of 6.00 - 8.00 log cfu/g and 26.67% of sucuk samples at the level of > 8.00 log cfu/g. Lactic acid bacteria constitute the dominant flora in fermented sausages without heat treatment such as sucuk and their numbers vary between 10^6-10^8 cfu/g (Gökalp et al., 2004; Kaban, 2013). In a previous research, it was reported that total aerobic mesophilic bacteria count in sucuk varied between $3.0x10^4$ - $2.2x10^8$ cfu/g (mean $2.9x10^7$ cfu/g) (Çon et al., 2002). Kaban and Kaya (2008) detected more than $1x10^8$ cfu/g lactic acid bacteria number in all of the sucuk samples examined. In a study conducted by Sancak et al. (1996), it was reported minimum, maximum and mean lactic acid bacteria counts as 1.7×10^6 cfu/g, 2.4×10^9 cfu/g and 3.3×10^8 cfu/g, respectively. In the current study, 10% of the samples had less than 4.0 log cfu/g of lactic acid bacteria and 13.33% of the samples had lactic acid bacteria counts between 4.0 - <6.0 log cfu/g for sucuk (Figure 2a). This result indicates that these products were subjected to a heat treatment. Indeed, it is stated in the regulation that this product is a fermented meat product without heat treatment (Anonymous, 2019). In heat-treated sucuk, 40% of the samples varied between 2.0 - <4.0 log cfu/g and 40% varied between 4.0 - <6.0 log cfu/g. In this product, only 3.3% of the samples had > 8.0 log cfu/g of lactic acid bacteria count. These results are thought to be due to the difference in the heat treatment parameters applied in the firms, and also the formulation. Lactic acid bacteria are microorganisms that contribute significantly to both product safety and sensory properties by producing acid during fermentation (Gökalp et al., 2004).

Table 2. Lactic acid bacteria, *Micrococcus/Staphylococcus*, yeast-mould and Enterobacteriaceae counts of the brands with respect to the fermented sausage type

Brand	Heat-treated Sucuk				Sucuk			
	LAB	MS	EB	YM	LAB	MS	EB	YM
A	7.38	4.23	$<10^2$	$<10^2$	3.80	5.30	$<10^2$	$<10^2$
	7.60	3.30	$<10^2$	$<10^2$	7.88	$<10^2$	$<10^2$	$<10^2$
	7.77	3.85	$<10^2$	$<10^2$	6.93	4.70	$<10^2$	$<10^2$
B	4.11	$<10^2$	$<10^2$	$<10^2$	2.48	3.94	$<10^2$	$<10^2$
	5.30	4.60	$<10^2$	$<10^2$	7.43	5.60	$<10^2$	$<10^2$
	7.85	4.69	$<10^2$	$<10^2$	7.90	5.20	$<10^2$	$<10^2$
C	3.65	7.85	$<10^2$	3.66	8.23	5.96	$<10^2$	$<10^2$
	3.69	2.95	$<10^2$	$<10^2$	6.78	5.70	$<10^2$	$<10^2$
	4.69	3.00	$<10^2$	$<10^2$	7.30	4.00	$<10^2$	$<10^2$
D	2.60	$<10^2$	$<10^2$	$<10^2$	8.30	3.30	$<10^2$	$<10^2$
	4.65	$<10^2$	$<10^2$	$<10^2$	8.30	4.78	$<10^2$	$<10^2$
	3.00	6.90	$<10^2$	$<10^2$	8.78	5.71	$<10^2$	$<10^2$
E	2.60	$<10^2$	$<10^2$	$<10^2$	8.52	4.85	2.47	$<10^2$
	4.40	$<10^2$	$<10^2$	$<10^2$	6.39	2.30	$<10^2$	$<10^2$
	2.48	$<10^2$	$<10^2$	$<10^2$	7.35	3.00	$<10^2$	$<10^2$
F	3.48	$<10^2$	$<10^2$	$<10^2$	7.00	$<10^2$	$<10^2$	2.60
	4.60	3.30	$<10^2$	$<10^2$	6.60	$<10^2$	$<10^2$	$<10^2$
	3.60	3.30	$<10^2$	$<10^2$	8.54	3.30	$<10^2$	$<10^2$
G	2.47	3.60	$<10^2$	$<10^2$	4.77	$<10^2$	$<10^2$	$<10^2$
	2.85	3.77	$<10^2$	$<10^2$	3.60	$<10^2$	$<10^2$	$<10^2$
	5.14	3.74	$<10^2$	$<10^2$	4.30	$<10^2$	$<10^2$	$<10^2$
H	7.90	6.08	2.69	$<10^2$	6.60	3.00	$<10^2$	3.17
	8.10	5.69	2.00	$<10^2$	4.85	$<10^2$	$<10^2$	3.60
	4.60	4.30	$<10^2$	$<10^2$	4.30	$<10^2$	$<10^2$	2.60
J	4.88	5.00	$<10^2$	$<10^2$	8.48	$<10^2$	$<10^2$	$<10^2$
	4.77	4.98	$<10^2$	$<10^2$	7.69	$<10^2$	$<10^2$	3.48
	2.00	3.93	$<10^2$	$<10^2$	7.81	$<10^2$	$<10^2$	$<10^2$
K	4.97	3.90	$<10^2$	$<10^2$	7.90	5.30	$<10^2$	$<10^2$
	4.08	3.92	$<10^2$	$<10^2$	8.48	4.95	$<10^2$	$<10^2$
	2.30	3.60	$<10^2$	$<10^2$	7.71	4.54	$<10^2$	$<10^2$

LAB: Lactic Acid Bacteria (log cfu/g); MS: *Micrococcus/Staphylococcus* (log cfu/g); EB: Enterobacteriaceae (log cfu/g); YM: Yeast-Mould (log cfu/g)

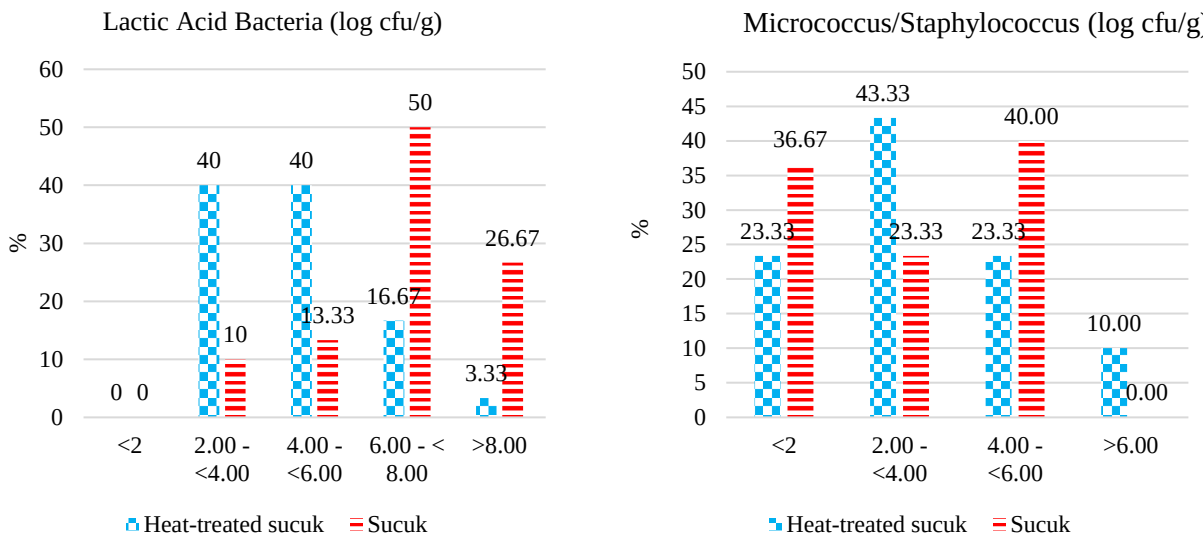


Figure 2. Frequency distribution of heat-treated sucuk and sucuk samples in terms of lactic acid bacteria and *Micrococcus/Staphylococcus* numbers

Micrococcus/Staphylococcus numbers of sucuk and heat-treated sucuk samples showed a large variation between brands, and even in different samples of the same brand. *Micrococcus/Staphylococcus* counts in all heat-treated sucuk samples of E brand were found below the detectable limit. In two samples of E brand and in one sample each of B and F brands, the number was also found below the detectable limit. As can be seen in Figure 2b, the number of *Micrococcus/Staphylococcus* was below the detectable limit in 23.3% of the heat-treated sucuk samples. This result is considered to be a good indicator that heat treatment is involved in the sucuk production. In addition, 40% of the samples gave *Micrococcus/Staphylococcus* counts of 4.0 - <6 log cfu/g in sucuk. Since *Micrococcus/Staphylococcus* are acid sensitive microorganisms, rapid acidification during fermentation can significantly inhibit the development of these products (Akköse et al., 2023). 23.3% of the sucuk samples gave *Micrococcus/Staphylococcus* counts of 2.0 - <4 and, 36.6% gave *Micrococcus/Staphylococcus* counts below the detectable limit (Figure 2b). These findings suggest that heat treatment is included in the sucuk process rather than a rapid acidification. Micrococci and staphylococci are technologically important microorganisms in these products and contribute to product quality with nitrate reductase and catalase activities. These microorganisms have also effects on flavour with their proteolytic and lipolytic activities (Kaya and Kaban, 2020).

In heat-treated sucuk, Enterobactericeae were detected at a level of 2 log cfu/g in two samples of only one brand (H), and the number was below the detectable number in the other samples. In sucuk, Enterobactericeae was detected only in 1 sample of E brand (Table 2).

These results show that some firms also apply heat treatment to the sucuk. Çon et al. (2002) reported that the number of Enterobacteriaceae in sucuk varied between <10 – $1.1x10^4$ cfu/g (mean: $1.3x10^3$ cfu/g). Kaban and Kaya (2008), on the other hand, found the Enterobacteriaceae count under the detectable limit in the samples of 5 brands (10 samples) and 10^2 cfu/g in two brands (4 samples).

Yeast-mould count of heat-treated sucuk was found below the detectable limit in all samples of the other brands except C. In sucuk, the number of yeast-mould was determined at the level of 10^2-10^3 cfu/g in all three samples of H brand. In addition, it was determined at the level of 10^2 cfu/g in 1 sample of F brand and 10^3 cfu/g in the sample of J brand (Table 2). Çon et al. (2002) determined the number of yeast-mould in sucuk between <10 – $1.4x10^5$ cfu/g (mean: 1.2×10^4 cfu/g) and Sancak et al. (1996) determined between 10^2-10^6 cfu/g (mean: 10^5 cfu/g). In the research conducted by Atasever et al. (1998), it was also determined as 6.4×10^4 cfu/g. In our study, the fact that the number of yeast-mould in sucuk is generally below the detectable number indicates the application of heat treatment in sucuk production.

Conclusion

pH is an important criterion for sucuk and heat-treated sucuk. The mean pH value was found below 5.4 in all sausage samples obtained from different firms. The pH value of the heat-treated sucuk is also below the limit value (5.6) in all except one brand (B). However, in terms of a_w value, a wide variation was detected in both the sucuk and heat-treated sucuk. In sucuk, the mean a_w value is below 0.90 in only one brand (D). In another brand (K), the mean a_w value was found to be 0.905. In other brands, high a_w values were determined, including 0.95. These results indicate that these products were not subjected to adequate drying process. In heat-treated sucuk, the a_w value varied between 0.928-0.957. However, the residual nitrite level in all samples was less than 15 mg/kg. Enterobacteriaceae and yeast-mould counts in heat-treated sucuk and sucuk were generally below the detectable limit. Lactic acid bacteria counts were <6 log cfu/g in 23.33% of the sucuk samples and <6 log cfu/g in 80% of the heat-treated sucuk samples. In 23.3% of the sucuk samples, the *Micrococcus/Staphylococcus* count was 2 - <4 log cfu/g, while in 36.6%, the count was below the detectable limit. These findings suggest that heat treatment was applied in

the sucuk production process. On the other hand, considerable differences were obtained between different batch numbers of the same product in some brands. This demonstrates that these firms do not produce in a standardized manner.

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Acute and Subacute Toxicity of *Ruta Montana* Extract to Female Rats: Effect on Liver, Kidneys and Ovaries

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ABSTRACT

Ruta montana L. is an annual aromatic plant of the family rutaceae. Quantitative analysis of the methanolic crude extract of *Ruta montana* L. yielded 8.43%, whereas the qualitative analysis revealed the presence of alkaloid or coumarin. The Litchfield and Wilcoxon method calculated the LD_{50} of the crude methanolic extract of *Ruta montana* L. in Wistar albino female rats at 393.18 mg/kg. This allows the plant to be classified as moderately toxic. The subacute toxicity study of the methanolic crude extract of *Ruta montana* L. in female Wistar albino rats treated with 100 mg/kg (≈ 1/4 LD_{50}) and intraperitoneally showed a significant increase in body weight of the rats treated at the 4th week. Animals treated and sacrificed after 30 days showed a disturbance of the relative mass of the organs. Biochemical parameters of hepatic function assessment showed a significant increase in PAL with elevation of AST and ALT, whereas those of renal function revealed a significant decrease in creatinine with an increase in urea. Hematologic parameters recorded a decrease in RBC, HGB and HCT. The histological sections of the treated rats reveal the existence of blood congestion in the central veins and liver tissues, foci of necrosis and steatosis in the liver, blood congestion and some glomerular atrophy in the kidneys, as well as blood congestions and developed follicles without oocytes in the ovaries.

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Introduction

Plants and their extracts are indispensable sources of life, used for the prevention and treatment of human diseases in many countries (Selamoglu, 2018). Among these plants are those of the Rutaceae family, which includes the annual aromatic plant Ruta montana L. (Quezel and Santa 1963). Rutaceae are frequently woody plants having secretory pockets of a sort not found in any other so-called schizolysigenic family (Ozenda, 2000). *Ruta montana*, *Ruta chalepensis*, *Ruta tuberculata*, and *Ruta latifolia* are the four species found in Algeria. They differ from one another in terms of the morphology of their leaves, fruiting clusters, bracts, and sepals (Quezel and Santa, 1963).

Ruta montana L. thrives in warm and temperate climates. It naturally thrives on arid slopes and rocks. It is common in the Mediterranean basin's calcareous soils (Quezel and Santa, 1963), as well as in arid regions of southern Europe and North Africa. It can be found in desert grasslands and mountainous regions in Algeria (Clevely and Richmond, 1997).

This plant contains above the phytochemicals compounds such as alkaloids, flavonoids, essential oils, coumarins and phenols, which make it widely used in traditional medicine (kara et al., 2016). *Ruta montana* L. was employed in ancient Greece and Egypt to induce abortions where it is used as an emmenagogue, an aphrodisiac, abortive (Masri et al., 2015; Polio et al., 2008). Root decoction is used to treat liver disorders, pulmonary diseases and stomach ailments (Lahsissene et al., 2009). To induce abortion, it is ingested in the form of paste or decoction (Bellakhdar, 1997). The objective of this work is to assess the acute toxicity of the methalonic extract through the determination of LD_{50} and the effect of subacute toxicity on liver, kidney and ovaries.

Materials and Methods

Plant Material

The plant *Ruta montana* L. was collected in the northern region of the Setif City, north-east Algeria, when it reached maturity in late spring and early summer during the flowering and fruiting period. The identification of the plant was done on the basis of its morphological characteristics and confirmed by Dr. Nouiwa Wafa

(Department of Plant Biology and Ecology, Faculty of Nature and Life Sciences, Setif 1 University, Algeria).

A specimen has been deposited at the Faculty of Natural and Life Sciences. The aerial components were cleaned, then dried at ambient temperature and shielded from light in a ventilated area (Figure 1).

Figure 1. The plant Ruta montana L. (2019)

Plant Extraction

Using an electric grinder, the aerial parts of *Ruta montana* L. are ground. A good extraction yield by maceration is possible by increasing the surface area of the powder obtained with the extraction solvent. 711 g of the plant powder of the aerial part is macerated in 1000 mL of methanol (99.7%).

A vacuum filter is used to filter the heterogeneous mixture after it has been mechanically stirred for 72 hours at room temperature. The methanol in the filtrate is allowed to evaporate naturally in the open air. The recovered *Ruta montana* L. crude methanolic extract (EBMRM) is kept in the fridge until usage.

Thin layer chromatography (TLC).

Before experimenting with EBMRM in animals, we checked whether or not active ingredients such as alkaloids which have a strong toxic action in the extract. A ready-to-use TLC plate made of Macherey-Nagel 60 F254 silica gel, 20 x 20 cm in size, was used after drying. The mobile phase prepared is a mixture of methanol/chloroform/ammonia: 78.5/20/1.5 (V/V/V) (Kurt, 1971).

After dissolving the 1g of EBMRM in 1 mL methanol, a drop is placed 1 cm from the bottom of the TLC plate and then dried in an oven. The plate is immersed about 0.5 cm in the mobile phase contained in a conventional glass tank whose atmosphere will have previously been saturated with vapors of the mobile phase, which then progresses by capillarity along the stationary phase, and entrains the compounds contained in the EBMRM according to their weight and their solubility. When the front of the solvent reaches 3 cm from the upper edge, the plate is removed from the tank, then dried and revealed by spraying with Dragendorff's reagent to reveal the presence of alkaloids which are known to be toxic.

Acute Toxicity

Animals

Adults female rats (albinos Wistar) weighing about 210–245 g were purchased from the Pasteur institute (Algiers-Algeria). The animals were acclimatized for 3 weeks to the conditions of the animal room of the faculty of Nature and Life Sciences, Setif 1 University before the commencement of the study. They were fed a standard diet and tap water *ad libitium;* however the litter was changed two to three times a week. For easy identification, the rats were marked on their body by a solution of picric acid (2%).

In the absence of an ethics committee on the use of animals for scientific purposes in our University, Ethical approval for the study was sought from the scientific council of the Faculty of Natural and Life Sciences-Ferhat Abbas University Setif 1. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals.

Determination of the lethal dose (LD_{50})

Lethal dose 50 (LD_{50}) is the amount of a chemical that, when given to laboratory animals, results in the death of 50% of them (Diallo, 2005). The LD_{50} makes it easy to gauge a substance's toxicity and define toxicity classes (Oduola et al., 2007). By marking the rats, female rats weighing are separated into five groups of six each. Prior to the experiment, the animals fasted for 24 hours. One dose of 250, 400, 600, 1000, or 1100 mg/kg of rat weight of the test substance is injected intraperitoneally to the treated rats after being dissolved in a few drops of methanol and diluted in physiological water. A saline solution with a trace amount of methanol was given to the control group. The animals are individually monitored for the first day and every day for 14 days following the administration of EBMRM. The number of dead rats as well as the behavior and clinical symptoms of the animals are noted throughout the experiment.

The LD_{50} and its confidence interval are calculated using the Litchfield and Wilcoxon method (Litchfield and Wilcoxon, 1949).

Subacute toxicity

20 rats were divided into two groups of 10 animals each: the treatment group and the control group. The second batch received 0.5 mL of saline solution (0.9% NaCl), while the first batch received 100 mg/kg (≈1/4 LD50 mg/kg) of EBMRM intraperitoneally. For a total of 30 days, animals receive daily treatment under subacute toxic circumstances.

Study of Some Hematological and Serum Biochemical Parameters

The animals were sedated by chloroform inhalation under a bell at the conclusion of the experiment, and after 30 days of intraperitoneal administration of EBMRM and physiological saline, blood samples were obtained, conducted with hematocrit tubes at the level of the orbital vein of the animal's eyes.

Blood from each animal was collected in heparinized tubes for the measurement of biochemical parameters and ethylene diamine tetra-acetic acid (EDTA) tubes for the determination of hematological parameters namely RBC (red blood cells), WBC (white blood cells), HCT (hematocrit), HGB (hemoglobin), PLT (platelets), MGV (mean corpuscular volume), MPV (mean platelet volume), MCHC (mean corpuscular hemoglobin concentration) which were performed using a α Swelab Coulter blood cell counter.

The heparinized tubes were spun at 3200 rpm for 5 min in order to measure the biochemical factors glucose, urea, and creatinine using the Spinreact clinical diagnostic reagent kits - Barcelona Spain. For aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (PAL) were used the Tunisian Society's Biomaghreb enzyme kits (BIOMAGHREB) - Tunis Tunisia. The animals are sacrificed, dissected, and their organs macroscopically examined *in situ* after blood collection; after that, they are removed and weighed on a precision scale.

Histological Study

The liver, kidneys, and ovaries of the control and treatment rats were rapidly removed and stored in 10% formalin. Fixation, dehydration, incorporation in paraffin, creation of 5µm slices, staining with hematoxylin-eosin (HE), and observation under an optical microscope are the many stages of microscopic examination.

Statistical analyzes

The "Sigma Stat 3.5" program was used to conduct the statistical analysis. The one-way ANOVA test was used to statistically assess the data, which are represented by the mean and standard deviation. *P values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Ruta montana L. aerial components were extracted by maceration, and the resulting 8.43% yield crude extract took the form of a dark green sticky paste. After being revealed by Dragendorff's reagent, the alkaloids or coumarins that appeared in the form of an orange-colored spot could be separated using thin layer chromatography of the crude methanolic extracts of the aerial portions of *Ruta montana* L. (Figure 2).

Acute Toxicity

Observation of animal behavior and clinical symptoms

From the beginning of treatment with various doses of crude methanol extract from the airborne portions of *Ruta montana* L. administered intraperitoneally to female rats, toxic effects were visible. The clinical map displayed by the animals was marked by severe symptoms, including convulsions and tremors, tachycardia, hair smoothing, and abdominal intussusception. The animals begin to become less active, move more slowly, and end up lying on their belly, with their back legs spread out.

Following the injection of a dose of 1100 mg/kg of EBMRM, the female rats died within the first few minutes.

The Rats that managed to survive in the treated groups did not eat and remained in a severe condition, but they started to improve gradually and eventually recovered from the second day.

Determination of the LD_{50} by the method of Litchfield and Wilcoxon.

Following the intraperitoneal administration of EBMRM to different groups of rats at different doses ranging from 250 mg/kg to 1100 mg/kg, the percentage and dose-dependent mortality of female rats in Probit units is presented in Table 1 and Figure 3. The sum of the various contributions to $\chi 2$: $\Sigma\ \chi 2$ experimental = 0.46 $\chi 2$ experimental = $\Sigma\ \chi 2$ experimental. N/K = 0.46.30/5 = 2.76 (N: total number of animals, K: total number of doses. The theoretical $\chi 2$ value for the probability threshold p = 0.05 for a degree of freedom n = 5-2 (the dose-2 numbers) is 7.82. It is therefore acceptable to work. From the data presented in the Table 1, we plot the Probit curve as a function of the logarithmic dose presented in Figure 3. The toxicity was observed to be a dose-dependent phenomenon.

From the line drawn, we have determined:
The LD_{16} = 145.26 mg/kg.
The LD_{50} = 393.18 mg/kg.
The LD_{84} = 1064.20 mg/kg.

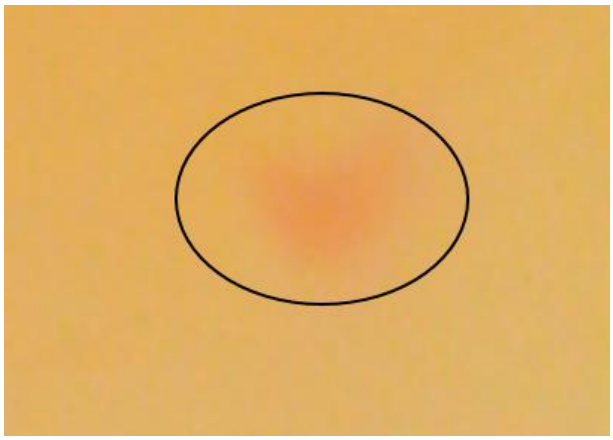

Figure 2. Chromatogram of the crude methanolic extract of *Ruta montana* L.

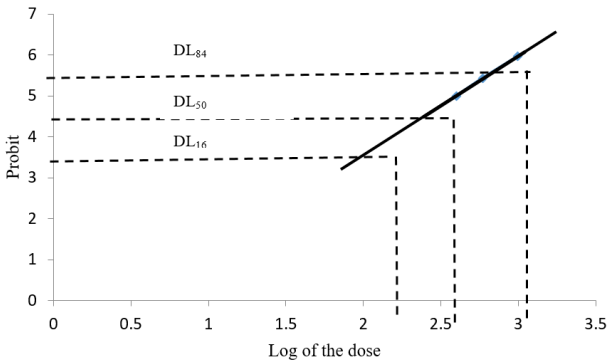

Figure 3. Curve characterizing the dose-response relationship for the determination of lethal parameters in female rat treated by simple application with the EBMRM of *Ruta montana.*

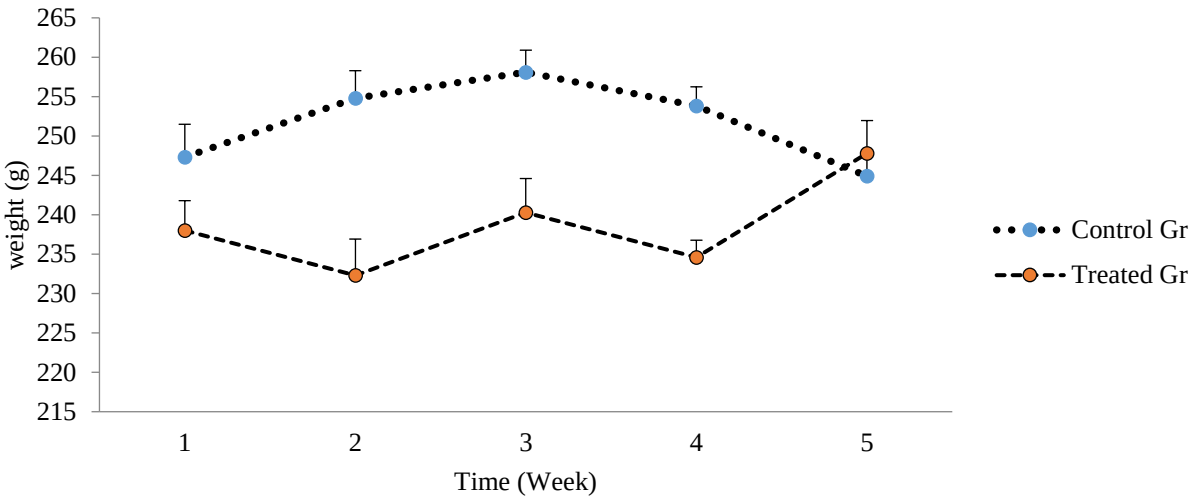

Figure 4. Changes in body weight (g) between the control and treatment rats after exposure to 100 mg/kg of EBMRM under subacute toxicity conditions

The values are shown as mean ± standard deviation, * significantly different, P<0.05. Time: 1- Initial weight; 2- 1st Week; 3- 2nd Week; 4- 3rd Week; 5- 4th Week.

Table 1. Calculus of median lethal dose of the EBMRM from *Ruta montana* using Litchfield and Wilcoxon method.

Dose mg/kg	Number of deaths/group	Log dose	Observed effect		Expected effect		Difference de	χ^2
			%	Probit	%	Probit	%	
250	0/6	2.39	7.8	3.58	27.9	4.41	20.1	0.23
400	3/6	2.60	50	5.00	43.3	4.82	6.7	0.02
600	4/6	2.77	66.6	5.43	56.9	5.17	9.7	0.04
1000	5/6	3	83.3	5.97	74.1	5.64	9.2	0.05
1100	6/6	3.04	93.9	6.55	79.7	5.82	14.2	0.12

Table 2. The relative mass of the organs of the control and treated rats under the conditions of subacute toxicity by the dose of 100 mg/kg of EBMRM.

Group	Lungs	Heart	Liver	Spleen	Kidneys	Brain
Control	0.00703±0.000735	0.00335±0.000292	0.0327±0.00356	0.00433±0.000623	0.00724±0.000676	0.00717±0.00109
Treated	0.00617±0.000454 *	0.00324±0.000217	0.0349±0.00169	0.00410±0.000773	0.00679±0.000829	0.00723±0.000681

The values are shown as mean ±standard deviation, * significantly different, P≤ 0.05.

Weight fluctuation

The difference in body weight between the treated and control rats is seen in Figure 4. Weight changes were minimal in the first and third weeks, somewhat positive in the second, and significantly positive in the fourth for the treated group. The first and second weeks saw typical weight gain for the controls, followed by a modest reduction in the third and fourth weeks.

The relative mass of the organs

By comparing EBMRM-treated rats with macroscopically observed controls, the organs showed no visible morphological changes. With the exception of a noticeable reduction in the relative mass of the lungs, no significant difference was seen between the relative masses of the treated rats' organs and those of the control group presented in Table 2.

Study of hematological parameters

The hematological results obtained are illustrated in Table 3. A significant increase in mean blood cell volume (MMV), red blood cell distribution index (IDRa) and a significant decrease in mean corpuscular hemoglobin concentration (CCMH) have been registered. Values for the other parameters showed no significant difference presented in Table 3.

Study of serum biochemical parameters

Rats given EBMRM showed slightly elevated levels of ASAT and ALAT as well as a considerable increase in PAL when blood biochemical parameters were examined to assess the condition of the liver and kidneys which are presented in Figure 5. With a slight increase in urea and glucose shown in Figures 6 and 8, serum creatinine concentrations in treated rats decreased significantly in Figure 7.

Histopathological study

It became possible to see some glomerular atrophy and blood congestion in the kidneys of rats treated with EBMRM under subacute toxic circumstances compared to the control rats by looking at histological sections of the kidneys presented in Figure 8.

In contrast to the control rats, the treated rats' livers showed foci of necrosis, hepatic steatosis, and blood congestion in the central veins and hepatic tissues presented in Figure 9. It was able to see thrombus, developed follicles without oocytes, and others with oocytes with a very low quantity of oocytes in the ovaries of the treated rats through histological sections presented in Figure 10.

Table 3. Hematological parameter values of control and treated rats when exposed to subacute toxicity at a dose of 100 mg/kg EBMRM.

Parameters	RBC 10^{12}/l	VGM fl	IDR %	IDRa Fl	HCT %	PLT 10^9/l	VPM Fl	IDP fl	PTC %	LPCR %
Control	7.470 ± 0.231	56.083 ± 1.165	14.400 ± 0.410	39.117 ± 1.105	41.917 ± 1.808	678.333 ± 118.867	5.700 ± 0.245	9.183 ± 0.279	0.388 ± 0.0741	3.550 ± 0.860
Treated	7.128 ± 0.347	58.383 ± 1.286*	14.767 ± 0.734	43.067 ± 1.665*	41.650 ± 2.468	690.333 ± 93.712	5.667 ± 0.216	9.033 ± 0.234	0.390 ± 0.0623	3.483 ± 0.866
Parameters	GB10^9/l	HGBg/dl	TCMHpg	CCMHg/dl	LYM10^9/l	GRAN1$0^9$/l	MID10^9/l	LYM %	GRA %	MID %
Control	5.917 ± 1.516	13.400 ± 0.473	17.917 ± 0.204	31.967 ± 0.463	4.117 ± 1.023	1.167 ± 0.561	0.633 ± 0.367	70.600 ± 9.725	20.167 ± 7.256	9.233 ± 3.552
Treated	5.550 ± 1.719	12.817 ± 0.722	17.950 ± 0.524	30.750 ± 0.481*	3.783 ± 0.868	1.133 ± 0.592	0.633 ± 0.432	69.650 ± 8.734	20.533 ± 6.287	9.817 ± 3.205

Les valeurs sont présentées en moyenne ± écart type, * significativement différent, P<0.05.

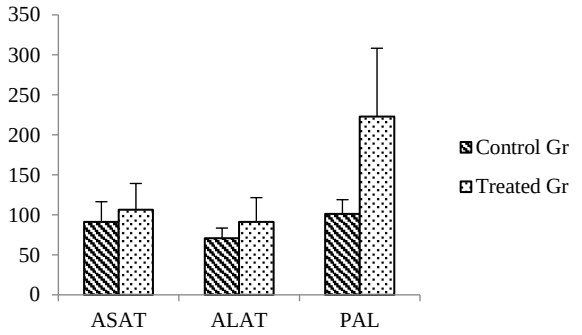

Figure 5. Serum level of "ASAT, ALAT and PAL" of control and treated rats in the conditions of subacute toxicity by the dose of 100 mg/kg of EBMRM.
Values are expressed as mean ± standard deviation, * significantly different, P < 0.05.

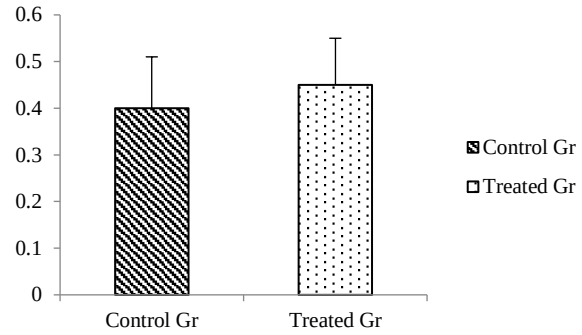

Figure 6. Serum "Urea" levels of control and subacute treated rats under conditions of subacute toxicity conditions with 100 mg/kg EBMRM.
Values are expressed as mean ± standard deviation.

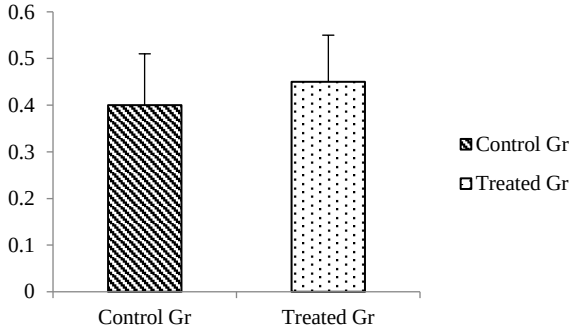

Figure 7. Under subacute toxicity circumstances, serum "creatinine" levels of untreated and treated rats received a dose of 100 mg/kg of EBMRM.
Values are presented as mean ± standard deviation, * significantly different, P < 0.05.

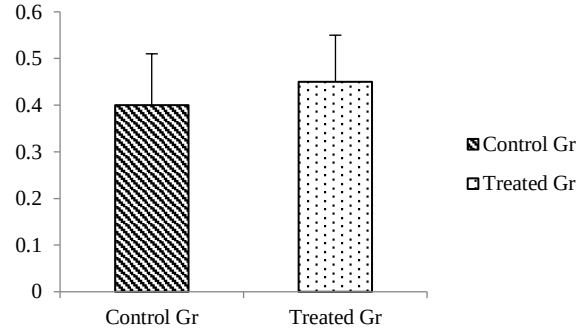

Figure 8. Serum "glucose" levels of control and treated rats were measured under subacute toxic circumstances with a dosage of 100 mg/kg EBMRM.
Values are expressed as mean ± standard deviation

Discussion

The Rutaceae family includes the plant *Ruta montana* L. (Ozenda, 2000). It is well known for its pharmacological effects (Da Silva et al., 2006; Pelletier, 1983), as well as its toxic and particularly anti-fertilizing effects, an emmenagogue, anaphrodisiac and abortive (Masri et al., 2015;Ulubelen et al., 1994).

An 8.43% yield was obtained by extracting the dry powder from the *Ruta montana* L. plant by macerating it in methanol. This yield is lower than that obtained by Ghadjati *et al*. whose raw extract yield was 12.42% (Ghadjati et al., 2022).This variation is likely due to several possible causes, including the maceration period, the chemical composition of the plant, which may vary

depending on the location and time of collection, the age of the plant parts, the nature of the soil, the climate (Brown et al., 2012; Özcan and Chaichat , 2005), as well as the extraction solvent used (Kalt et al., 2001).

The TLC allowed for the separation of several EBMRM constituents, and the Dragendorff reagent revealed the presence of an orange spot, which is likely a coumarin or an alkaloid because coumarins can also produce a weak, non-specific reaction with the reagent (false positive Dragendorff reaction) because of the α,β unsaturated lactone structure (Wagner and Bladt, 1996). This result is not in agreement with the work of Ghadjati et al. where they separated three alkaloids from an extract of total alkaloids of *Ruta montana*, and Touati et al. where they identified 6 alkaloids from *Ruta montana* collected in Morocco using more developed methods like Heteronuclear multiple quantum coherence (HMQC), Heteronuclear Multiple Bond Correlation (HMBC) and MS spectral (Touati et al., 2000). This could be mainly explained by the more developed methods used, the more humid climate and the nature of the soil in Morocco.

Female rats treated with EBMRM under acute toxic conditions displayed a clinical profile that was marked by an accelerated heart rate, which was most likely brought on by the blockage of muscarinic M2 receptors, which resulted in the suppression of vagal tone. The central nervous system (CNS) has likely been damaged by preventing the generation of acetylcholine in the synapses of the CNS, which results in respiratory distress, leg paralysis, and convulsions (Goulle et al., 2004). The majority of the survivors regained a normal appearance up to day 14, while the probable causes of death were respiratory arrest and convulsions. These results are in agreement with those obtained by Ghadjati (Ghadjati *et al*., 2022). The LD_{50} of treated female rats is equal to 393.18 mg/kg following treatment with EBMRM using the Litchfield and Wilcoxon technique in acute toxicity settings. The plant *Ruta montana* L. can be categorized as moderately poisonous using Hodge and Sterner's toxicity categorization system (Frank, 1991).

The first symptoms of a toxic substance are changes in body weight, food consumption and general behavior (Almança et al., 2011). Rats treated with a dose of 100 mg/kg under circumstances of subacute toxicity showed a minor loss of body weight in the first and third weeks, followed by a considerable gain in the fourth. The adverse effects of chemicals and drugs are related to changes in body weight. However, many experts agree that these changes in body weight are due to fat accumulation and physiological adaptation to the plant extracts rather than the toxic effects of the chemicals or drugs causing the animal to eat less because it has no appetite (Kifayatullah et al., 2015).

The relative mass of the organs is a good parameter for indicating whether the organ has been targeted by a drug or not (Hor et al., 2012). The calculation of the relative mass of the organs of the treated and control rats made it possible to detect a significant decrease of 12.58% in the lungs of the treated rats. According to Girish et al., a decrease in the relative mass of the lungs is probably due to pulmonary necrosis caused by the administration of chemical substances (Girish et al., 2009).

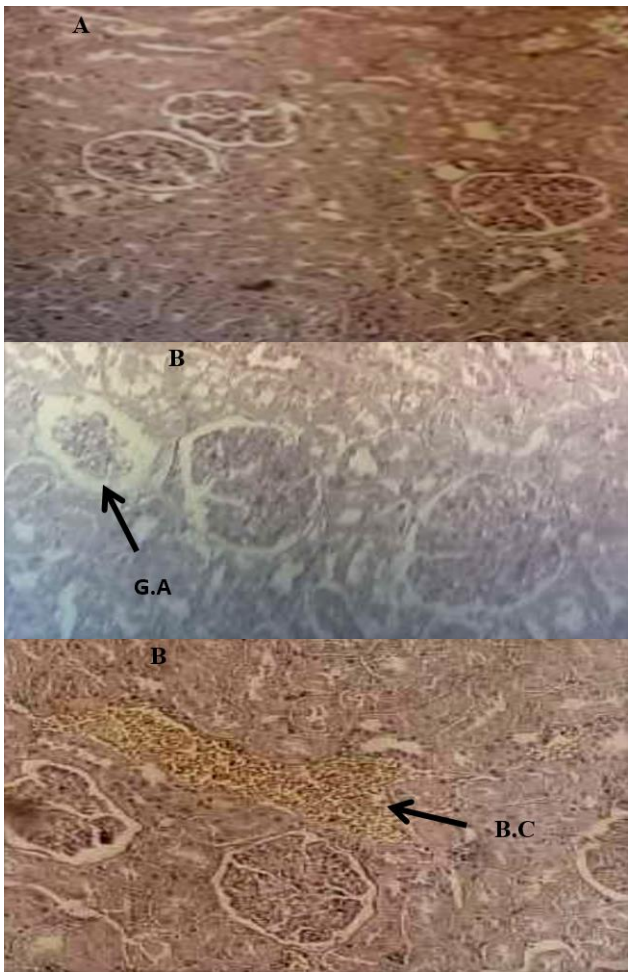

Figure 9. Histological sections of kidney tissue from control (A) and treated (B) rats under subacute EBMRM (100 mg/kg) toxicity conditions.
Glomerular atrophy (G. A) and blood congestion (B.C) (x100). Coloring HE

One of the most vulnerable systems to the effects of toxic substances is the hematopoietic system, which also serves as a crucial indicator of both pathological and physiological health in both humans and animals (Kulkarni et al., 2012). When data are extrapolated from animal research, analysis of blood parameters is important for risk assessment since any alteration in the hematological system is a great indicator of human toxicity (Chandra et al., 2012). MCV and IDRa levels in hematological parameters significantly increased whereas mean corpuscular hemoglobin concentration significantly decreased (MCHC). For the diagnosis of anemia, MCHC and MCV are crucial RBC indices (Voigt, 2000). Additionally, the MCHC parameter indicates the hemoglobin concentration of red blood cells while the VGM parameter gives details on the size and quality of erythrocytes (Nussey et al., 1995). However, the impact of EBMRM on RBCs, may contribute to the decrease in RBC, HGB, and HCT values. If the dosage (>100 mg/kg) or the frequency of the treatment are increased, it can be concluded that EBMRM may have an anemic effect on the blood system. The leakage of cellular enzymes into the plasma is definitely an indication of liver damage. A number of enzymes that are typically found in the cytoplasm of hepatocytes are released into the blood when their plasma membranes are damaged, and their detection in serum serves as a helpful indicator of the kind and severity of hepatotoxicity (Kumar et al., 2004).

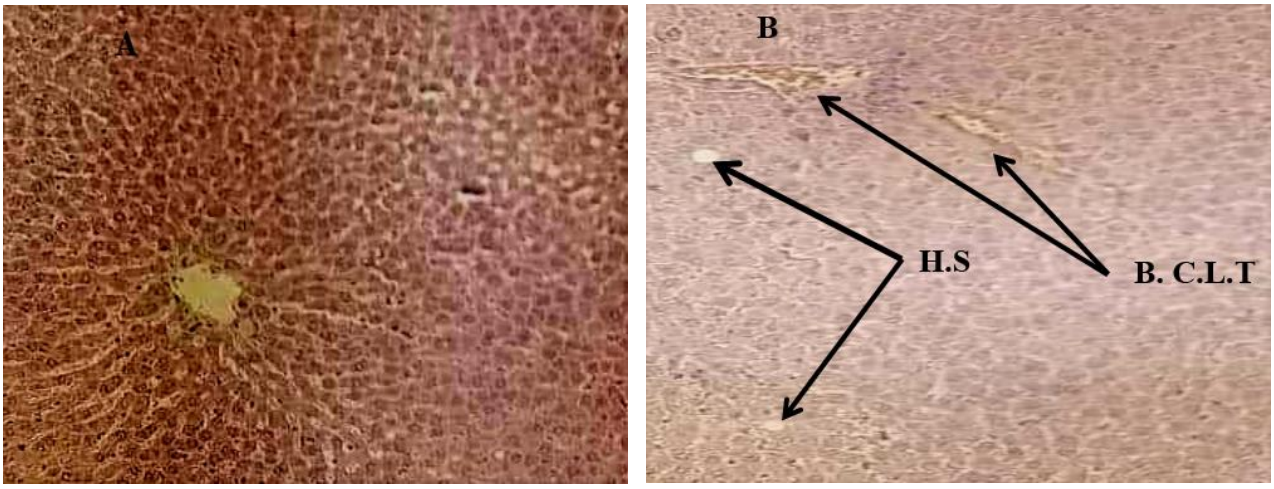

Figure 10. Histological sections of hepatic tissue from control rats (A) and rats treated (B) under the conditions of subacute toxicity by EBMRM (100 mg/kg).
B. C.L.T: Blood congestion in liver tissue. H. S: Hepatic steatosis. (x100). Coloring HE

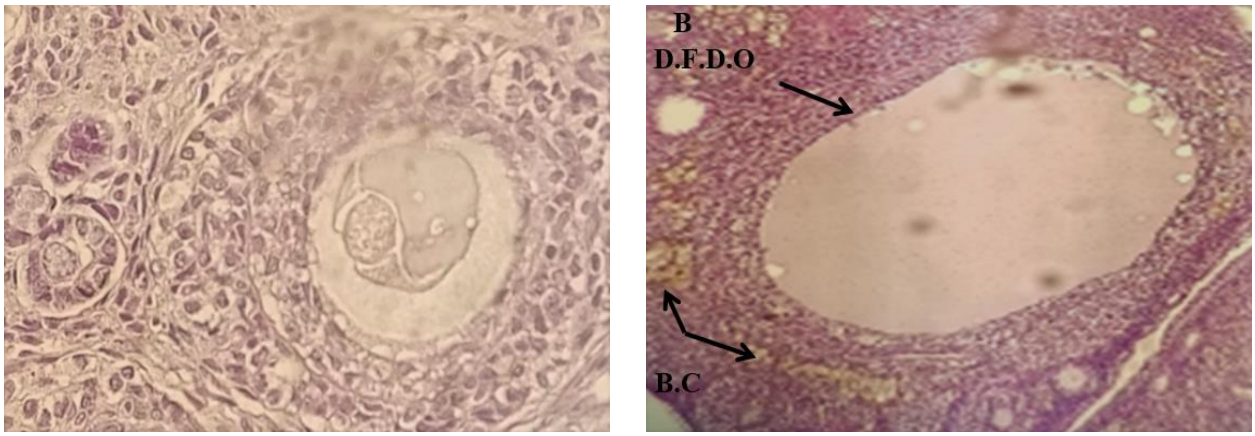

Figure 11. Histological sections of ovarian tissue from control (A) and treated (B) rats in the conditions of subacute toxicity by EBMRM (100 mg/kg).
D.F.D.O: Developed follicle devoid of oocyte. B.C: blood congestion. (x100). Coloring HE

Transaminases (ALAT and ASAT) are enzymes that have significant metabolic activity inside of cells; hence, an increase in their serum levels indicates cell damage, especially in the liver. The cytoplasmic enzyme ALAT is present in the liver in very high concentrations, and an increase in serum levels of this enzyme may indicate hepatocellular damage. But ASAT is an enzyme that is abundantly found in the mitochondria and cytoplasm of numerous organs, including the liver, heart, skeletal muscle, kidney, and brain (Magdy, 2013). Enzymes known as alkaline phosphatases (PAL) are found throughout the body, but are concentrated in the liver, bone, gut, kidneys, and white blood cells. Alkaline phosphatase is released as a result of damage to these organs (Lazare et al., 2011).

The assay of the biochemical parameters in the serum of the treated and control rats made it possible to detect a significant increase in the rate of PAL in the group of treated animals compared with the controls. The increase in PAL can be explained by damage to the bile ducts, i.e. cholestasis (Aragon and Younossi, 2010).

The existence of some hepatic necrosis seen in the histological sections can be used to explain a minor elevation in the serum levels of ALAT and ASAT. Hepatic steatosis, blood clots in the veins and liver tissues, and foci of necrosis were also found during a histopathological analysis of the liver sections. The latter are most probably brought on by an imbalance of lipid metabolism induced by intraperitoneal administration of EBMRM. Additionally, the biochemical analysis allowed for the validation of an increase in blood sugar. Although this could be a result of the stress-related increase in metabolic performance, it does not necessarily mean that there is a problem with glucose metabolism (Landray et al., 2002).

Concentrations of urea, creatinine and electrolytes in serum or urine are used to measure renal function. Serum creatinine is a reliable indicator of renal function (Atsamo et al., 2011).

When comparing treated and control rats, it was shown that treated rats had higher serum urea levels and significantly lower creatinine levels. The decrease in creatinine may reflect both skeletal muscle mass and physical activity level (Baxmann et al., 2008). This may also be explained by the fact that rats rapidly degrade and eliminate alkaloids and their metabolites (Hardman et al., 1998). An increase in the degradation of protein molecules may help to explain the rise in urea (Lullmann et al., 1998). Analysis of renal histological sections revealed some glomerular atrophy and a low level of renal blood congestion. The kidney continued to function normally despite the damage.

When subacute toxicity at a dose of 100 mg/kg was applied to rats, histological sections of their ovaries revealed that there were more mature follicles without oocytes than oocyte-containing follicles. These findings could be explained by how EBMRM affects meiosis, which occurs during oogenesis. These results are in agreement with those obtained by Ghadjati *et al.* (Ghadjati et al., 2022).

Conclusion

The *Ruta montana* L. plant, which is a member of the Rutaceae family, is reportedly harmful to both humans and animals, according to the bibliographic research. It was able to identify the crude methanolic extract of *Ruta montana* L. as a moderately toxic product due to the acute toxicity circumstances observed in female Albino Wistar rats. Several hematological and biochemical parameters were disturbed, including liver and kidney function, in rats given the crude methanolic extract of *Ruta montana* L. at a dose of 100 mg/kg (≈ 1/4 LD_{50}). Histological examination of the liver, kidney, and ovary indicated some structural abnormalities.

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Impact of Modern Beehive Technology Adoption on Household Income: Evidence from North Shewa Zone, Oromia National Regional State, Ethiopia

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ABSTRACT

Hidabu Abote, Dera, Wera Jarso and Debra Libanos districts of North Shewa zone are potential in honey production. To enhance this potential, different organizations disseminate improved beehives technologies for the smallholder farmers. However, the impact of the disseminated technologies on household income has not been evaluated. Thus, this study aimed to evaluate the impact of improved beehive adoption on household income. Purposive and two stage sampling technique was used to select 384 sampled households. The study used logistic regression model to identify the determinants of adoption decision of modern beehive technology while propensity score matching to evaluate the impact of modern beehive technology adoption on household income. The result of logistic regression model shows that age of household head, family size, households experience in beekeeping, frequency of extension contact, access to credit services, access to training and access to beehive demonstration site visit had positive and significant effect on household adoption decision of modern beehive technology. The result of propensity score matching indicates that the adopters of improved beehive technology were earned Birr 2690.383 than non-adopter. The difference in household income between the two groups shows that there is considerable room for improvement of household income through increasing the number of adopter of improved beehives technology in the study area. This should be done through provision of training, credit, extension and expansion of beehive demonstration site among the others.

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Introduction

Agriculture is a basis for the entire socioeconomic structure of the country and has a major influence on all other economic sectors and development processes and hence, plays a crucial role in poverty reduction in Ethiopia (CSA, 2021). Even if the share of agriculture to GDP is reduced recently, it has still largest contributions i'e 35% to the total country GDP, 73% to employment and 90% to foreign exchange earnings (CSA, 2021). Moreover, the livelihood of about 90% of the poor is fully or partly dependent on agriculture as a result of which, agricultural development will continue to be the basis for economic growth and development.

Ethiopia has huge potential of the apiculture sub-sector, which holds a key position for poverty reduction and natural resource conservation in the country (MoA and ILRI, 2013). Despite of its contribution for smallholder households' income in particular and nation's economy in general, honey production system is very traditional which results in low productivity and poor quality. Thus, the government of Ethiopia has amplified its attention to develop the apiculture sub-sector as one of its strategies for poverty reduction and different NGOs have been intervening to assist the poor smallholder farmers through introduction and promotion of improved beehives technologies to obtain higher production and good quality that can enable the smallholder farmers to be benefited from the sub-sector. To increase the production and productivity of honey and bee wax, different improved technologies have been used in the last 7-10 years in the country (MoA, 2015).

Oromia National Regional State government of Ethiopia under its agricultural led development policy gave due attention to apiculture development in selected areas of the region based on their prioritized potential. To develop this potential and increase production from the sector, different improved beehive technologies have been introduced. Even though large number of modern beehive technologies have been introduced and promoted by the regional bureau and other non-governmental organizations over the past 10 years, however, the amounts of modern beehive technologies used by farmers were very limited (Akinwumi et al., 2001).

In the study area (North Shewa zone) livestock and fishery department disseminated various modern beehive technologies solely and in collaboration with different projects. However, there is no compiled and tangible information regarding the impact of modern beehive technology adoption on household income in the study area. Therefore, this study aimed to evaluate the impact of modern beehive technology adoption on household income in the study area.

Research Methodology

Sampling Techniques and Sample Size Determination

The target populations of this study were honey producer households in the study area. Purposive and two stage stratified random sampling techniques were employed to select sample respondents. Among 13 districts found in North Shewa zone, 4 districts namely Hidabu Abote, Dera, Debra Libanos and Wera Jarso were purposively selected based on honey production potential. After that, a total of 8 kebeles were purposively selected based on honey production potential. Total household head in the sample kebeles were stratified into two groups (adopters & non-adopters of modern beehive technology). Finally, 192 adopters and 192 non-adopters households were randomly selected for interview. The sample size was determined using the Cochran (1977) formula specified in equation 1.

$$n = \frac{Z^2 P(1-P)}{D^2} = 384 \quad (1)$$

Where:

n = sample size;
Z = the table value of 95% confidence interval=1.96
P = the population proportion (assumed to be 0.5 for it provides the maximum sample size)
D = degree of accuracy expressed as a proportion (0.05)

Types of Data and Method of Data Collections

Both primary and secondary data were used. Primary data was collected using structure questionnaires. Key informant interview was conducted to supplement primary data. Besides, secondary data was collected from zone and district livestock and fishery office.

Method of Data Analysis

The study was used descriptive statistics, inferential statistics, and econometrics model to analyze the collected data.

Descriptive and inferential analysis

Descriptive statistics like mean, standard deviation, and percentage were used for describing the socioeconomic and institutional characteristics of sample households in the study area. Chi-square test and independent sample t-test were employed to compare the adopters and non-adopters households in terms of the hypothesized covariates.

Econometric Analysis

The two commonly used discrete choice models in the adoption studies are the probit and logit models. The results from the two models are very similar since the normal and logistic distributions from which the models are derived are very similar except for the fact that the logistic distribution has slightly fatter tails (Gujarati and Porter, 2009). The dependent variable which is normally used with these models is dichotomous in nature, taking the values 1 or 0, a qualitative variable which is incorporated into the regression model as dummy variable. This study was used binary logistic regression model to identify the factors affecting modern beehive technology adoption in the study area.

In most studies propensity score matching (PSM) method has been used to evaluate public policies or projects or programs. A PSM matches each technology adopter households with a non-adopter household that has almost the same likelihood of adopting any social programs to find the closest comparison group from a sample of non-adopters to the sample of modern beehive technology adopters. In impact estimation PSM constructs a counterfactual comparison group based on a model of the probability of participating in the treatment, using observed characteristics. Participants are then matched on the basis of this probability, or propensity score, to non-participants for the impact evaluation. Therefore, this study was used PSM model to evaluate the impact of modern beehive technology adoption on household income in the study area.

Common Support Region

The assumption is that P(x) (probabilities) lies between 0 and 1. This restriction implies that the test of the balancing property is performed only on the observations whose propensity score belongs to the common support region of the propensity score of treated and control groups (Becker and Ichino, 2002). Individuals that fall outside the common support region would be excluded in the treatment effect estimation. This is an important condition to guarantee improving the quality of the matching used to estimate the ATT (average treatment on treated). The ATT is simply the mean difference in outcomes over the common support, appropriately weighted by the propensity score distribution of participants.

Matching Quality Test

It helps to check if the matching procedure is able to balance the distribution of the relevant variables in both control and treatment group. The following common criteria were used to assess the matching qualities.

Standardized Bias: One suitable indicator to assess the distance in marginal distributions of the variables is the standardized bias (SB). For each covariate *X* it is defined as the difference of sample means in the treated and matched control subsamples as a percentage of the square root of the average of sample variances in both groups.

T-test: It is used to check if there are significant differences in covariate means for both groups (Rosenbaum and Rubin, 1983). Before matching differences are expected, but after matching the covariates should be balanced in both groups and hence no significant differences should be found.

Pseudo R^2: Sianesi (2004) suggests re-estimating the propensity score on the matched sample that is only on participants and matched non-participants and compare the pseudo-R^2's before and after matching. After matching there should be no systematic differences in the distribution of covariates between both groups and therefore, the pseudo-R^2 should be fairly low.

Definition of Variables and Hypothesis

Treatment variable: It is a dummy variable which takes value of 1 if the household adopted modern beehive technology and 0 otherwise.

Outcome variable: It is a continuous variable and defined as the amount of income household obtained from honey production in Ethiopian Birr.

Explanatory variables: The following explanatory variables were hypothesized to affect the adoption of modern beehive technology in the study area.

Results and Discussions

Characteristics of sample households in terms of categorical variables

Chi-square test was used to measure the relationship between adopter and non-adopter household in terms of the categorical variables. The result show that there was a statistically significant association between adopter and non-adopter households in terms of sex of household head, access to credit services, access to training, access to beehive demonstration site visit and types of farmer. It implies that male headed households, households who obtained credit, training, households who participated in beehive demonstration site visit and model farmers were more adopter of the improved beehive technology than non-adopter households at 1, 5 and 10% significance level (Table 2).

Characteristics of sample households in terms of continuous variables

A t-test was used to measure the mean difference of continuous variable between adopter and non-adopter households in the study area. The result show that there was statistically significant mean difference between adopters and non-adopters households in terms age of household head, family size, frequency of extension contact, beekeeping experience, and education level at 1% significance level (Table 3). This implies that older farmer; households who had large family members; large number of extension contact; more experienced farmers in beekeeping and more educated farmers were more adopter of improved beehive technology than non-adopter farmers.

Table 1. Summary of variables and hypothesis

Dependent variables	Types of variable	Measurement	Expected effect
Treatment variable			
Adoption of modern beehive technology adoption	Dummy	1 if adopter, 0 if not	
Outcome variable			
Total annual income household obtained from honey production	Continuous	Ethiopian Birr	
Explanatory variables			
1. Age of household head	Continuous	Years	+
2. Sex of household head	Dummy	1-Male; 0-Female	+
3. Educational level	Continuous	Years of schooling	+
4. Access to credit	Dummy	1-Used credit; 0-If not used	+
5. Extension contact	Continuous	Number of contact per year	+
6. Livestock size	Continuous	TLU	+
7. Family size	Continuous	ME	+
8. Experience in beekeeping	Continuous	Years	+
9. Types of farmer	Dummy	1 if model, otherwise (0)	+
10. Access to training	Dummy	1 if yes, otherwise (0)	+
11. Access to demonstration visit	Dummy	1 if yes, otherwise (0)	+
12. Access to market for honey	Dummy	1 if yes, otherwise (0)	+
13. Access to market information	Dummy	1 if yes, otherwise (0)	+

Table 2. Results of inferential analysis (chi-square test for categorical variables)

Variables		Adopters (n=192)		Non-adopters (n=192)		Total (n=384)		Pearson chi-square	
		Freq.	%	Freq.	%			X^2	P
Sex	Male	166	86.5	150	78.1	4.575	0.032**	4.575	0.032**
	Female	26	13.5	42	21.9				
Credit	Yes	126	65.6	59	30.7	46.823	0.000***	46.823	0.000***
	No	66	34.4	133	69.3				
Information	Yes	110	57.3	97	50.5	1.771	0.183	1.771	0.183
	No	82	42.7	95	49.5				
Training	Yes	126	65.6	50	26.0	60.587	0.000***	60.587	0.000***
	No	66	34.4	142	74.0				
Demonstration	Yes	116	60.4	59	30.7	34.111	0.000***	34.111	0.000***
	No	76	39.6	133	69.3				
Market	Yes	159	82.8	155	80.7	0.279	0.597	0.279	0.597
	No	33	17.2	37	19.3				
Type of Farmer	Model	23	12.0	12	6.3	3.804	0.051*	3.804	0.051*
	Ordinary	169	88.0	180	93.8				

***, ** and * denote significance at the 1, 5 and 10%, respectively; Source: Survey result (2022)

Table 3. Results of inferential analysis (t- test for continuous variables)

Variables	Adopters (n=192)		Non-adopters (n=192)		Independent sample t-test	
	Mean	Std. Err.	Mean	Std. Err.	t-value	P value
Age	46.71	0.43	37.44	0.44	-14.9	0.000***
Family size	7.55	0.08	4.60	0.10	-24.2	0.000***
Livestock size	5.69	0.16	5.87	0.17	0.50	0.747
Extension contact	8.02	0.18	3.70	0.11	-20.9	0.000***
Beekeeping experience	8.05	0.12	4.76	0.09	-21.9	0.000***
Education level	0.62	0.07	0.15	0.03	-2.60	0.000***

*** denote significance at the 1%; Source: Survey result (2022)

Table 4. Honey production among adopter and non-adopter households

Variable	Modern hive (n=192)		Traditional hive (n=192)		t-test	
	Mean	Std. Err.	Mean	Std. Err.	t-value	P value
Honey production	18.02	1.78	7.33	1.17	69.61	0.000***

Source: Survey result (2023); *** denote significance at the 1%

Honey Production Status among Adopter and Non-adopter Households'

The amount of honey produced per hive per kilogram using traditional and modern beehive was compared using independent sample t-test. The result in Table 4 shows that there was statistically significant mean difference in the amount of honey produced from traditional and modern hive at 1% significance level.

Determinants of Modern Beehive Technology Adoption in the Study Area

To identify factors affecting modern beehive technology adoption, binary logistic regression was employed. The logistic regression output stated in Table 4 revealed that age of household head, family size, households experience in beekeeping, frequency of extension contact, access to credit services, access to training and access to beehive demonstration site visit were significantly and positively influenced the adoption of modern beehive technology in the study area. The details of each explanatory variable are discussed as follow:

Age of household head: As expected, age of household head had positive and significant effect on household's modern beehive technology adoption at 10% significance level. The result indicated that as the age of household head increase by one year, the odd ratio of being adopter of the modern beehive technology would increase by 1.07 units. This is because older farmers are assumed to have gained knowledge and skill over time and hence, would able to evaluate technology than younger farmers. This result is supported by the finding of (Mignouna et al., 2011; Kariyasa and Dewi, 2011).

Family size: As expected, this variable influenced household adoption of modern beehive technology positively and significantly at p<1%. The result revealed that, other things remains constant, the odds ratio of being adopter of the technology was about 2.31 times greater for households with large family size than household with low family size. This is due to the fact that farmers with large family size might adopt the technology to satisfy the need of their family. This result is consistent with the findings of (Musa et al., 2016 and Sisay et al., 2013).

Farmers experience beekeeping: Household's experience in beekeeping had positive and significant effect on the adoption of modern beehive technology at 10% significance level. The result indicate that as household experience in beekeeping increase by one year, the odd ratio of being adopter of the modern beehive technology would increase by 1.34 units. This is due to the fact that experience would improve farmers' skill and awareness on honey production. Previous studies by Chilot et al. *(*1996); Abadi et al. *(*1999) also confirmed that experience of the household heads in beehive would positively affect adoption of modern technology.

Frequency of extension contact: This variable influenced household adoption of modern beehive technology positively and significantly at p<1%. This revealed, other things remains constant, the odds ratio of being adopter of the technology was about 2.04 times greater for households with access to extension services than households without such services. This is due to the fact that farmers who had access to extension services would be more progressive in adoption of improved beehive technology. This result is consistent with empirical findings of (Kassa et al., 2018).

Access to credit: This variable influence household adoption of modern beehive technology positively and significantly at p<5%. The result revealed that, other things remains constant, the odds ratio of being adopter of the technology was about 3.83 times greater for households with access to extension services than households without such services. This is because farmers who had access to credit would be able to buy modern beehive equipment than the others. This result is consistent with the finding of (Sisay et al., 2013; Workneh, 2017).

Access to training: This variable influenced household adoption of modern beehive technology positively and significantly at p<5%. The result revealed that, other things remains constant, the odds ratio of being adopter of the technology was about 3.37 times greater for households with access to training than households without training. This is due to the fact that training might have inculcated technical competency, more exposure to the subject matter and convinced to adopt the improved technologies in the farms. This result is consistent with empirical findings of (Rahman, 2007).

Access to beehive demonstration site visit: As expected, demonstration site visit had positive and significant effect on household's modern beehive technology adoption at 5% significance level. The result indicated that as household

access to demonstration site visit increase by one unit, the odd ratio of being adopter of the modern beehive technology would increase by 3.80 units. This is due to the fact that visiting apiary sites of other beekeepers or demonstration site help the farmers to develop his/her insight in beekeeping and positive perception towards an innovation or a new technology. Study by Tamrat (2015) also confirmed that farmers' participation in field days and demonstration enhance adoption of farm technology.

Results of Propensity Scores Matching

The propensity score for a given household was estimated using logit model where the dependent variable is adoption status and taking different covariates as independent variables. The estimated propensity scores lies between 0.1381 and 0.9189 with mean value of 0.6127 for adopter households while 0.1381 and 0.8632 with mean value of 0.3873 for non-adopter households (Table 6).

Table 5. Binary logistic regression model output

Adoption status	Odds Ratio	Std. Err.	Z	P>Z
Sex	0.696209	.6024738	-0.42	0.676
Age	1.073555	.0441426	1.73	0.084*
Family size	2.310692	.4970559	3.89	0.000***
Experience	1.341721	.215385	1.83	0.067*
Education level	1.305688	.5839794	0.60	0.551
Livestock size	0.8358022	.0983069	-1.52	0.127
Extension contact	2.040419	.3673729	3.96	0.000***
Access to credit	3.834241	2.314219	2.23	0.026**
Access to information	0.6913105	.398726	-0.64	0.522
Access to training	3.371929	2.011492	2.04	0.042**
Demonstration	3.800892	2.292785	2.21	0.027**
Access to market	1.368178	.9738025	0.44	0.660
Types of farmer	2.507421	3.253501	0.71	0.479
Constant	3.17e-07	7.69e-07	-6.16	0.000
Logistic regression	Number of obs.	= 384		
	LR chi2 (13)	= 426.72		
	Prob > chi2	= 0.0000		
Log likelihood = -52.808528	Pseudo R2	= 0.8016		

***, ** and * denote significance at the 1, 5 and 10%, respectively; Source: Survey result (2022)

Table 6. The distribution of propensity scores

Descriptions	N	Mean	Std. Dev.	Min	Max
Adopter	192	0.6127	0.2348	0.1381	0.9189
Non-adopter	192	0.3873	0.1838	0 .1381	0.8632
Total sample households	384	0.5000	0.2389	0 .1381	0.9189

Source: Survey result (2022)

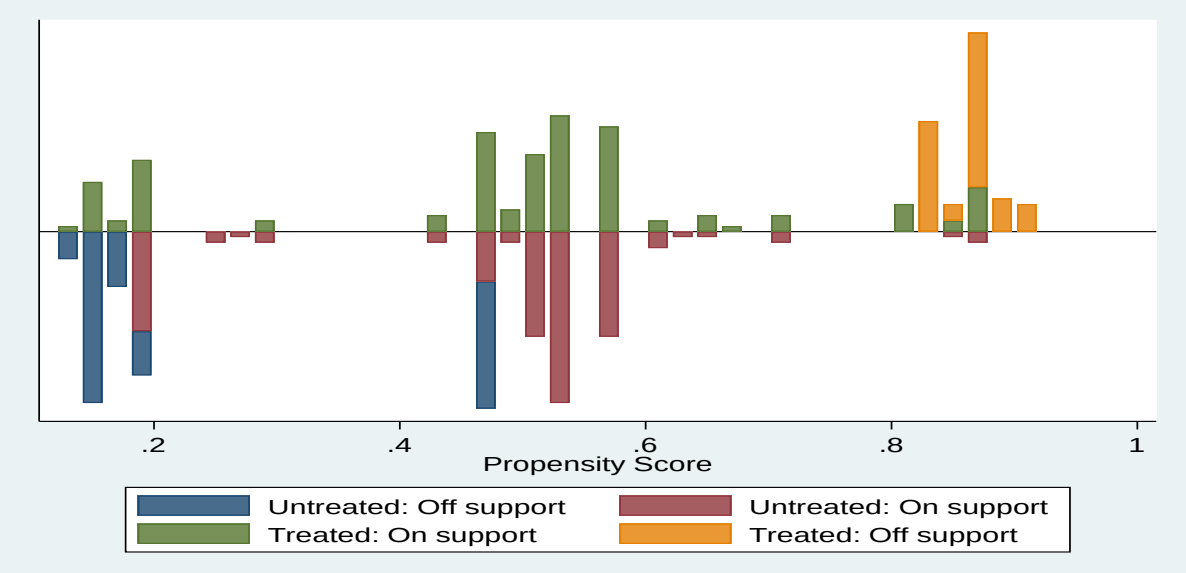

Figure 1. The distribution of propensity scores for treated and untreated groups
Source: Survey result (2022)

Table 7. Matching Quality Test

Covariates	Mean		% Bias	t-test	
	Treated	Control		t	p>t
Access to credit	.49231	.54206	-10.6	-0.76	0.448
Access to information	.56923	.69159	-19.5	-1.94	0.530
Demonstration visit	.41538	.33645	16.6	1.24	0.214
Access to market	.77692	.79439	-4.5	-0.32	0.746
Types of farmer	.09231	.09346	-0.4	-0.03	0.976
Ps R^2	LR Chi^2	$P>Chi^2$	Mean Bias		Med Bias
0.018	6.01	0.422	9.5		7.6

Source: Survey result (2022)

Table 8. The average treatment effect of matched adopter and non-adopter households

Outcome variable	Treated	Control	Difference	t-value	P-value
ATT	4544.2307	1853.8461	2690.3846	59.55	0.000***

Source: Survey result (2022); ***stands for statistical significance at 1%

Table 9. Sensitivity Analysis

Gamma	$e^{\gamma=1}$	$e^{\gamma=1.25}$	$e^{\gamma=1.5}$	$e^{\gamma=1.75}$	$e^{\gamma=2}$	$e^{\gamma=2.5}$	$e^{\gamma=2.75}$	$e^{\gamma=3}$
Sig+	0.00032	0.00058	0.0011	0.0025	0.0034	0.0062	0.0076	0.0084

Source: Model result (2022)

Common Support Region

As suggested by Bernard et al (2007) in order to ensure maximum comparability of the adopter and non-adopter households, the sample used for matching is restricted on those households who are located in the common support region. The common support region is where the values of propensity scores of both adopter and non-adopter groups can be found. The basic criterion of this approach is to delete all observations whose propensity score is smaller than the minimum of treated group and larger than the maximum of control group (Caliendo and Kopeinig, 2008). Based on the minima and maxima criterion, the region of common support is [0.1381, 0.8632] implying that the two groups share the same characteristics in these interval. Based on this criterion, 139 observations (77 from control and 62 from treatment groups) were discarded from the analysis (Figure 1).

Matching Quality Test

The pseudo R^2, t-test and standard bias are the basic tool for testing the quality of matching between treated (adopter) and control group (non-adopter). Low pseudo R^2, insignificant t-test after matching and standard bias below 20% is the universally accepted criteria to judge the quality of matching between adopter and non-adopter group (Rosenbaum and Rubin, 1983). As the result depicted in Table 7 shows that the pseudo R2 (0.018) was low, t-test value was insignificant and standard bias is below 20% (9.5%) for the selected covariates. This implies that the quality of matching was good to balance the characteristics in the treated and matched comparison group.

Results of Average Treatment Effect

The average treatment effect on treated (ATT) measures the average difference of income between the matched adopter and non-adopter households. The result of this study shows that the mean difference in total annual income between adopter and non-adopter households between the two groups of sample households is significant at 1% significance level. The average income gain due to adoption of the modern beehive technology adoption was Birr 2690.383 implying adopter households on average gain Birr 2690.383 more as compared to non-adopter (Table 8).

Results of Sensitivity Analysis

In order to overcome the unobserved bias, a Rosenbaum bounds calculation was used (sensitivity test) for the outcome effect on modern beehive technology adoption which is positive and significantly different from zero. A result in the Table 9 reveals that the inference for the effect of modern beehive technology for both the groups remains same and has been allowed to differ in their probability to being treated 1 up to 3 with unobserved covariates. It implies that p-critical values of the entire outcome e^{γ} (Gamma) is log odds of differential due to unobserved factors where Wilcoxon significance level for each significant outcome variable is calculated. Values which is corresponds to each row of the significant outcome variables are p-critical values (or the upper bound of Wilcoxon significance level) at different critical value of variables are found significant which are estimated at various level of critical value of e^{γ}. This further indicated that the study considered important covariates that affected both household adoption of modern beehive technology and outcome variable. On the basis of these results, the study concluded that average treatment on treated (ATT) impact assessment are found insensitive to unobserved selection bias and is an absolute effect of modern beehive technology adoption.

Conclusions and Recommendations

This study aimed to evaluate the impact of modern beehive technology adoption on the income of households in the selected district of Oromia National Regional State, Ethiopia. The study employed two stage sampling techniques to select 384 sampled households. Chi-square test, t-test, logistic regression model and propensity score

matching were employed to analyze the data. The result of chi-square test shows that there is statistically significant association between being adopter households and male headed household, access to training, access to beehive demonstration site visit and model farmer. Moreover, the result of t-test shows that there is statistically significant mean difference between adopter and non-adopter households in terms of age of household head, family size, beekeeping experience, frequency of extension contact, and education level of household head. This implies that there is a positive and significant relationship between being adopter of the technology and older farmer, large family size, having more experience in beekeeping, more frequency of contact with extension worker and attaining more education. The result of logistic regression revealed that frequency of extension contact, access to training, access to credit, age of household head, family size, beehive demonstration site visit and beekeeping experience had positive and significant effect on the household adoption decision of modern beehive technology. In addition, the results of propensity score matching indicate that households who were adopted modern beehive technology earned more income than non-adopter households. In order to increase the income of households' from honey production the concerned bodies should give due attention on how to expand modern beehives for smallholder farmers in the study area. This could be through improvement of credit services, extension services, training, experience sharing and expanding beehive demonstration site.

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Seroprevalence of Bovine Leukemia Virus Infection in Cattle in Muş Province, Türkiye

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ABSTRACT

Bovine leukemia virus (BLV) is known as the causative agent of enzootic bovine leukosis (EBL), which is a worldwide distributed disease and has also been detected in marketed beef and dairy products. BLV causes significant economic losses due to the loss of milk and yield or the slaughter of animals without adequate development. It has been reported in epidemiological studies that this infection is common in Türkiye, especially in the western provinces. There is no data on the possible presence or prevalence of BLV infection and its seroepidemiology in Muş province. The aim of this study is to determine the possible presence and prevalence of the infection, its role in yield losses, and to obtain epidemiological data on cattle farming in the Muş district. For this purpose, 300 blood serum samples were collected from cattle aged six months and older in the province of Muş and its different districts. The blood serum samples taken were tested for the presence of BLV-specific antibodies by agar gel immunodiffusion (AGID) and competitive enzyme-linked immunosorbent assay (C-ELISA) methods. As a result of the study, all of the controlled districts were evaluated as negative in the AGID and C-ELISA tests for the presence of BLV-specific antibodies. In conclusion, for the first time, it was demonstrated that cattle farming in the Muş province were BLV-free during the sampling period. Although BLV seropositivity was not detected in the tested animals, it is emphasized that the control of infection and eradication program should not be ignored.

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Introduction

Bovine leukemia virus (BLV) is known as one of the important viruses of bovine lymphotropic virus infection and is the etiological agent of enzootic bovine leukosis (EBL), also known as bovine leucosis (Rovnak et al., 1991). BLV, directly and indirectly, causes economic losses in cattle farms, such as a decrease in animal productivity, early slaughter, and restrictions on the import of animals and animal products from BLV-infected areas. Dairy cattle are the natural hosts for BLV infection, but buffaloes and camels can also become naturally infected (Nishimori et al., 2016).

The etiological agent is BLV, an exogenous C-type oncovirus located in the *Retroviridae* family, in the *Deltavirus* genus. It is closely related to human T-lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2). This virus preferentially infects B lymphocytes but has also been detected in T-lymphocytes, monocytes, and granulocytes (Marawan et al., 2021). This virus, as an RNA virus, upon entry into a cell rapidly creates a DNA copy of its genome by the enzyme reverse transcriptase in the host cell, and this retrotranscribed DNA copy predominates in infected cells (Ruiz et al., 2018).

BLV infection is a worldwide-distributed retroviral disease and is highly prevalent in North and South America, Asia, and Eastern Europe. Although most European countries have become free of infection through an efficient eradication program, the prevalence of disease remains high worldwide. The prevalence of BLV in the Middle East was lower than in other districts of the world, except for Türkiye and Iran, with an average of 48.3% and 64.7%, respectively (Rodríguez et al., 2011). The disease is listed by the World Organization for Animal Health (OIE) as an important disease for international trade (Ruiz et al., 2018).

The most important sources for BLV infection are blood lymphocytes and other tissue products of infected cattle (Mekata et al., 2015). The infection is not only transmitted horizontally, such as by arthropods and

iatrogenic transmission, but can also be transmitted vertically by ingestion of colostrum from BLV-infected cows or in utero. Iatrogenic transmission may occur through surgical instruments or sleeve gloves contaminated with infected blood during rectal palpation (Esteban et al., 2009). Most BLV-infected cattle (approximately 70%) do not show any clinical symptoms, of which about 30% develop persistent lymphocytosis (PL), and 1-5% of infected cattle develop malignant B-cell lymphosarcoma causing EBL (Pandey et al., 2017). Lymphoma occurs in approximately 5-10% of BLV-infected cows, predominantly in animals older than 3-5 years (Gutiérrez et al., 2014).

In Türkiye, clinical and pathological cases of leukosis were detected in dairy cattle for the first time in 1942 (Burgu et al., 1990). In the following years, the presence of infection was reported in serum and milk samples taken from dairy cattle from different regions of Türkiye, with seroprevalence rates varying between 0 and 59.6% (Çabalar et al., 2001; Otlu et al., 2001; Gülaçti et al., 2004; Özgünlük et al., 2005; Kale et al., 2007; Avcı et al., 2013; Acar and Gür, 2013; Şimşek et al., 2017; Ayvazoğlu et al., 2021).

BLV infected cattle develop specific antibodies against the major core protein p24 and envelope (gp51) virion proteins in their serum and milk; therefore, antibody-based serological tests are widely used for the diagnosis and screening of BLV infection in cattle older than 6 months and are a good indicator of the disease (Constable et al., 2016). Among serological tests, ELISA and AGID are the reference techniques that are recommended by the OIE for the diagnosis of BLV infection through the detection of antibodies that are directed to BLV gp51 and p24 proteins. Since there is no effective vaccine against the disease, control of the disease is not possible. Therefore, detection and early diagnosis of the disease are of great importance in order to reduce the spreading and the economic losses it causes (Marawan et al., 2021). Eradication schemes for BLV infection were mainly based on serological diagnosis by the AGID test, followed by the separation or removal of infected animals. Although AGID is the gold standard, ELISAs have been frequently used due to their higher sensitivity. The AGID test is a specific but not very sensitive test for detecting antibodies in serum samples from individual animals. However, the relatively low sensitivity of AGID may result in the occurrence of low-titer BLV infections in clinically normal herds (Dolz and Moreno, 1999; OIE, 2012; Marawan et al. 2021). In retroviral infections, ELISA is a rapid method and is used as a diagnostic and screening test for BLV because of its high sensitivity and specificity (Mousavi et al. 2014; Elhaig et al., 2017).

Data on the seroprevalence of BLV infection in cattle are essential not only in countries that have been successful in eradication programs and have already made some progress in controlling the disease but also in countries that need to establish a strategy for eradication programs. Therefore, the present study was aimed to determine the seroprevalence of BLV antibodies in cattle in Türkiye based on the AGID test (IDVet, BLV AGID, France) and Bovine Leukosis Serum C-ELISA (ID Screen® BLV Competition, France).

Material and Methods

Ethical Statement

The study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (approval date: 27/01/2022; decision no: 2022/01-02) and the Ministry of Agriculture and Forestry of the Republic of Türkiye (approval date and no: 17/01/2022-4144507).

Study Area and Sampling

The current study was carried out in the province of Muş and its five different districts located in the Eastern Anatolia region of Türkiye (Figure 1). A total of 300 serum samples were collected randomly from healthy-looking cattle over 6 months old from private livestock farms between March and October 2022 to determine the presence of BLV anti-gP51 antibodies (Figure 2). All serum samples of cattle collected into vacuum tubes were centrifuged at 2000 rpm, and the sera transferred to the stock tubes were inactivated at 56˚C for 30 minutes in order to inactivate indigenous complement and kept at -20˚C until testing. Sera samples were tested with an BLV-AGID kit and a competitive ELISA-Ab kit reported and validated in the previous study using the same commercial test kits to detect BLV anti-gP51 antibodies (OIE, 2012). The AGID and C-ELISA assays were performed according to the manufacturer's instructions.

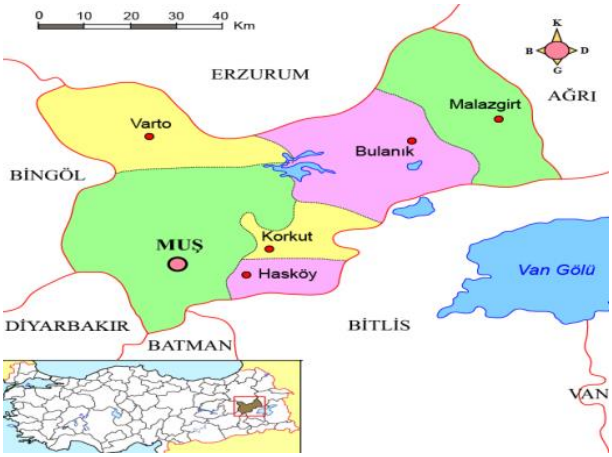

Figure 1. Geographical map of Muş province in Türkiye
The red circles show the location of Muş province and the districts where the cattle samples were collected

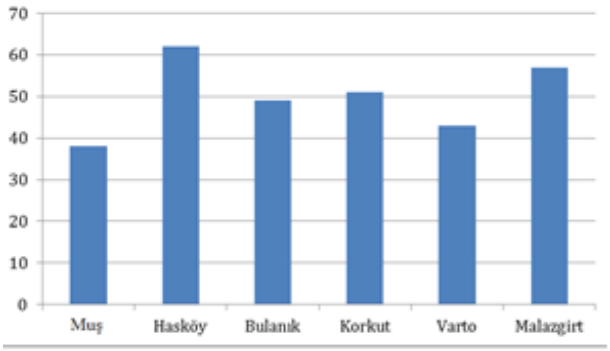

Figure 1. Distribution and number of serum samples collected in this study based on districts

Table 1. The number of samples based on districts and their serological results in AGID and C-ELISA methods

District	Number tested	BLV-AGID +	BLV-C-ELISA +
Muş (center)	38	-	-
Hasköy	62	-	-
Bulanık	49	-	-
Korkut	51	-	-
Varto	43	-	-
Malazgirt	57	-	-
Total	300	0%	0%

Results and Discussion

In the present study, antibodies against BLV infection were not detected in all 300 serum samples obtained from cattle from different districts of Muş province at ages ranging from 6 months to 10 years in either method by using BLV-AGID and BLV C-ELISA, as summarized in Table 1. The results of this study reveal that there was no virus circulation in cattle in Muş province during the sampling period.

Table 1. The number of samples based on districts and their serological results in AGID and C-ELISA methods

BLV infection is widespread all over the world except in western Europe (Rodríguez et al., 2011; Morovati et al., 2012). In Türkiye, infection was first reported in 1942 (Burgu et al., 1990) and studies on the seroepidemiology of the infection have been reported by many researchers using AGID and ELISA techniques in serum samples obtained from cattle (Otlu et al., 2001; Çabalar et al., 2001; Kale and Öztürk, 2004; Yildirim and Burgu, 2005; Özgünlük et al., 2005; Yavru et al., 2007; Yildirim et al., 2008; Acar and Gür, 2013; Ayvazoğlu et al., 2021). In the eastern neighboring countries, the prevalence rate of BLV infection has been reported to be between 0.5-25% in Iran (Morovati et al., 2012) and 7% in Iraq (Khudhair et al., 2016). Previous serological studies in Türkiye reported different seroprevalence rates for the presence of BLV antibodies.

The seropositivity of BLV infection was found to be 0% in cattle in the Kars region in 2001 (Otlu et al., 2001). In a study conducted in the Burdur region, the presence of BLV infection in dairy cows was found to be 4.9% in the AGID method and 19.18% in the ELISA method (Kale and Öztürk, 2004). In the Northeastern Anatolia region, BLV seropositivity rates were reported as 8% in Artvin, 4.87% in Erzurum, and 0% in Iğdır, Ağrı, Kars, Bayburt, Gümüşhane and Ardahan (Yildirim and Burgu, 2005). In the study carried out within the GAP (Southeast Anatolia Project), the AGID method was used on samples taken from cattle in nine provinces (Siirt, Diyarbakır, Batman, Adıyaman, Şanlıurfa, Gaziantep, Kilis, Mardin and Şırnak) in the Southeastern Anatolia region, 0.27% were positive for BLV antibodies (Özgünlük et al., 2005). The seroprevalence of BLV in culture-bred cattle owned by small-scale family farms in the Kars region has been reported as seronegative using AGID and ELISA methods (Yildirim et al., 2008). In the Afyonkarahisar in 2013, the rate of BLV positivity in cattle samples was determined to be 15.45%, and they reported that infection rates were low or even absent in small-scale family-type enterprises (Acar and Gür, 2013). In recent years, seropositivity has been reported as 0% in serum samples taken from cattle aged 1–10 years in Ardahan province (Ayvazoğlu et al., 2021). The data from these studies show that the seropositivity rates determined in private or public livestock where animal husbandry is carried out in large herds are much higher than the seropositivity rates in livestock in small family farms. In addition, the presence of BLV in cattle in the Eastern regions is lower and/or 0% compared to the Western and Central Anatolian regions of Türkiye.

In the current study, BLV seropositivity rate was found to be 0% in 300 blood serum samples collected randomly from cattle bred in different districts of Muş province by using AGID and ELISA. The results of this study are compatible with the results of previous studies conducted on BLV prevalence in the Eastern region of Türkiye (Otlu et al., 2001; Gülaçti et al., 2004; Özgünlük et al., 2005; Yildirim and Burgu, 2005; Yildirim et al., 2008; Acar and Gür, 2013; Şimşek et al., 2017; Ayvazoğlu et al., 2021). On the other side, the results of the current study showed compatibility with the results of previous studies that sampled animals on small family farms type in provinces close to Muş (Otlu et al., 2001; Özgünlük et al., 2005; Yıldırım and Burgu, 2005; Yıldırım et al., 2008; Ayvazoğlu et al., 2021).

Conclusion

Large animal livestock in the mentioned region is generally carried out in the style of small family farms for the purpose of livelihood. According to 2021 data, the total number of cattle in the region and its districts was reported as 328,207 heads. Therefore, it is important to reveal the epidemiological data and determine the control/eradication program based on the slow persistence and transmission routes of the infection. In this way, the status of BLV infection in cattle on small family farms in the region has been determined for the first time in terms of epidemiological dynamic information. In addition, although BLV seropositivity was not detected in the tested animals, it is thought to be useful to emphasize that a control/eradication program of BLV infection in animals bred on small family farms should be planned and initiated in advance.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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Callus Formation and Camphor Accumulation in Response to Sorbitol Stimulated Osmotic Stress in Yarrow

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ABSTRACT

Sorbitol is an important source of abiotic stress that is used to increase osmolality in cell cultures. It increases the antioxidant enzymes of defense catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the stress state of cells. Sorbitol plays an important role in stimulating these enzymes in cells and increasing phenylalanine ammonium lyase (PAL) activity. The aim of this study was to apply increasing doses of sorbitol elicitor to cell suspension cultures to determine the changes in cell number, viability, dry weight, and camphor content. *In vitro* plantlets were obtained from plant seeds and stem segments of these plants were used as explant source. Cell cultures were established after callus formation. Then, 0 (control), 5, 25, and 50 g L^{-1} sorbitol was dissolved in distilled water and cultured. Samples were taken three times in total, starting from day 1 to day 3. The content of camphor was detected by gas chromatography-mass spectrometry (GC-MS). Cell number, viability,dry weight, and camphor content increased significantly with increasing doses of sorbitol compared to sampling times. Compared to the initial culture, the amount of camphor increased by 40% at the 5 g L^{-1} dose, 82% at the 25 g L^{-1} dose, and 154% at the 50 g L^{-1} dose. In *A. gypsicola* cell cultures, increasing doses of sorbitol have clearly demonstrated the secondary metabolite accumulation and its positive effect on cell growth.

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Introduction

The *Achillea* genus, a significant member of the Asteraceae family, boasts a global representation of over 100 species. Within this genus, the essential oil harbors the camphor terpene, yielding medicinal benefits such as antimicrobial, antitussive, antinociceptive, antimutagenic, anticarcinogenic, and cardiovascular effects. Moreover, this versatile oil finds application in diverse industries including pesticide, cosmetics, plastics, and anti-rust coatings (Lin et al., 2007; Cheng et al., 2009; Sherkheli et al., 2009; Abdel-Rahman et al., 2015).

A dominant source of the camphor compound is the *Cinnamomum camphora* tree, renowned as the camphor tree, thriving in the far eastern regions. This tree comprises around 68% camphor in its chemical makeup (Frizzo et al., 2000). In contrast, the endemic *Achillea gypsicola* Hub, with its herbaceous form, exhibits a remarkable 61.8% camphor content (Açıkgöz, 2019). Studies demonstrate the *Achillea* genus's heightened camphor concentration compared to other medicinal and aromatic plants. Notable camphor contents among these species are: 0.6% (*Achillea filipendulina*), 1.3% (*A. magnifica*), 2.1% (*A.millefolium*), 3.17% (*A. aleppica*), 5.9% (*A. crithmifolia*), 6.7% (*A.santolina*), 7.1% (*A. tenuifolia*), 8.6% (*A.biebersteinii*), 15.92% (*A. tenuifolia*), 16.6% (*A. wilhelmsii*), 17.7% (*A. micrantha*), 22.8% (*A. grandifolia*), 23.21% (*A. magnifica*), 32.65% (*A. cucullata*), and 61.8% (*A. gypsicola*) (Pavlović et al., 2008; Smelcerovic et al., 2010; Toncer et al., 2010; Khiyari et al., 2014; Almadiy et al., 2016; Sampietro et al., 2016; Ahmadi-Dastgerdi et al., 2017; Ghasemi, 2017; Demirci et al., 2017; Açıkgöz, 2020b).

Considering its growth cycle, *A. gypsicola* emerges as a notable camphor-rich species within the plant kingdom, even rivaling tree forms (Açıkgöz, 2017). Ecological conditions significantly influence plants, resulting in diverse effects on the quality and consistency of their secondary metabolites. Stress plays a pivotal role in shaping the chemical composition of medicinal and aromatic plants. Plant stress is categorized into two groups: biotic and abiotic. Abiotic stress encompasses non-biological factors like physical, chemical, and hormonal influences. Physical stressors include light variations, UV

exposure, osmotic stress, drought, salinity, and thermal fluctuations. Osmotic stressors such as mannitol or sorbitol inhibit mineral absorption, slowing plant growth and development (Dodds and Roberts, 1985; Thompson et al., 1986). Various studies employ substances like polyethylene glycol (PEG) or sorbitol to simulate artificial drought stress, as these substances reduce osmotic potential, creating water stress non-metabolized by plants (Rai et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012).

Classical plant production methods and the extraction of secondary metabolites prove more resource-intensive and time-consuming compared to cell cultures. Hence, tissue culture techniques, particularly callus culture, emerge as popular methods for secondary metabolite production (Açıkgöz et al., 2018a; Açıkgöz et al., 2018b; Açıkgöz et al., 2019; Açıkgöz et al., 2022; Dağlioğlu et al., 2022; Ebru et al., 2022; Açıkgöz et al., 2023). Callus culture efficiently generates products of specific quality and standards, enhances genetic diversity, introduces new compounds absent in the mother plant, optimizes space utilization, and utilizes minimal resources. This study investigates the impact of abiotic stress through sorbitol elicitation on callus cultures, focusing on changes in cell number, viability, dry weight, and camphor content.

Materials and Methods

Plant Material

Seeds of *A. gypsicola* were procured from their native environment near the regions of Çorum, located in central Anatolia, Turkey. The Ministry of Food, Agriculture, and Livestock granted written permission for the collection. Validation of the species was carried out by Prof. Dr. Hayri Duman. The herbarium of the Field Crops Department at the Ordu University Faculty of Agriculture securely housed voucher plant specimens. Plant seeds were harvested by sampling from all plant clusters available in the area and were kept in storage until they were cleaned and planted in cork-stopped glass jars. The slope of the land where the plants were collected was 32.0–37.2%, the altitude 743–760 meters, and the aspect was determined as south-southwest (Figure 1).

Cultivation of Callus and Elicitation Sorbitol

In vitro plantlets derived from the collected seeds (pre-treatments) (Açıkgöz and Kara, 2019) served as the source explants (Figure 2). The establishment of *A. gypsicola* cell suspension cultures was initiated using callus tissues obtained from stem segments. The cultures were sustained in B5 medium, fortified with 0.5 mg L^{-1} of benzylaminopurine and 0.5 mg L^{-1} of naphthalene acetic acid. These cultures were maintained in three 250-mL Erlenmeyer flasks, each containing 50 mL of liquid medium and 2.5 g of delicate green calluses. Incubation was carried out on a rotary shaker at 105 rpm, with the temperature set at 25 °C and a photoperiod of 16 hours light/8 hours dark. Three concentrations (5, 25, and 50 g L^{-1}) of sorbitol were tested, alongside the control groups receiving equivalent volumes of ethyl alcohol and distilled water, respectively. The cell suspension cultures, following consistent incubation conditions as mentioned earlier, were harvested at 8-, 24-, and 48-hours post-elicitation. This aimed to assess the influence of sorbitol on cell growth, as well as the accumulation of camphor compounds. After aseptic filtration using Whatman No. 3 filter paper and subsequent washing with deionized water, the filtered suspension cultures were stored in a deep freezer at -20 °C for subsequent extraction. For chemical analysis, the cell suspension cultures were homogenized using a mortar and pestle. The extraction followed the methodology outlined by Açıkgöz (2021). Specifically, 2 g of suspension on a fresh weight basis were mixed with 10 mL of 96% ethyl alcohol and homogenized for 2 minutes. This mixture was maintained at 45°C in a water bath for one night. Subsequently, the samples were placed on a rotary shaker at 4000 rpm for 5 minutes, and the resulting supernatant was collected in vials. The collected extracts were evaporated fully dry at 75 °C using a rotary evaporator. The dried residues were dissolved in 1 mL of methanol for subsequent chemical analysis (Açıkgöz, 2021).

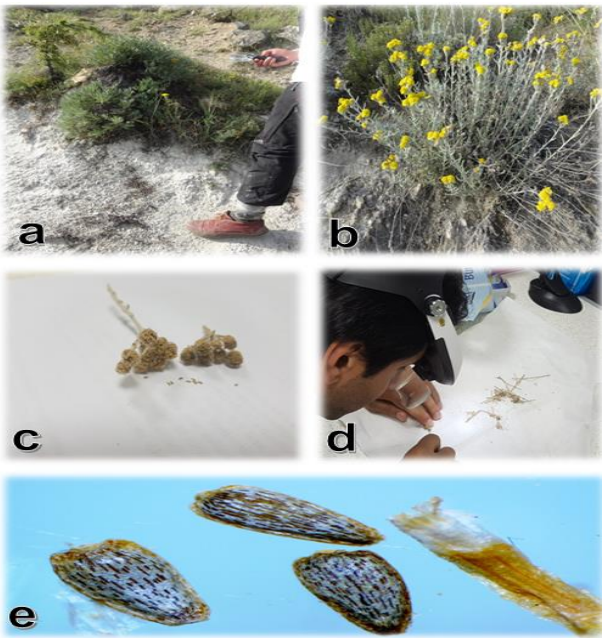

Figure 1. Image of the soil structure of *A. gypsicola* species
(a), view of the plant in the field (b), image of seeds in the laboratory (c), image of pest control (d) and view of seeds under a binocular microscope (e)

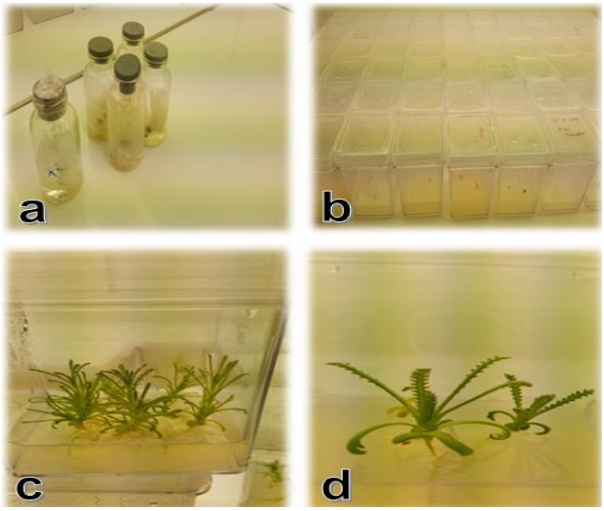

Figure 2. Image of the pre-treatments of *A. gypsicola* species
(a) and images of *in vitro* plantlets germinating in the climate chamber in magenta pots (b, c, and d)

Assessment of Cell Dry Weight, Number, and Viability

The progression of cell suspensions was assessed by gauging key parameters encompassing cell dry weight (g L^{-1}), cell number, and cell viability (%). Cell dry weight was ascertained by carefully weighing the filtered cell samples, which had been exposed to a controlled temperature of 55 °C for 48 hours within an oven. This meticulous process allowed for the precise determination of cell mass. To evaluate cell viability, a Trypan Blue solution from Thermo Fisher Scientific, USA, was utilized in accordance with the methodology delineated by Laloue et al. (1980). This approach facilitated the differentiation between viable and non-viable cells based on dye exclusion. The quantification of cell numbers was carried out utilizing a Nageotte Counting Chamber (Hausser Scientific, USA), employing the procedure elucidated by Moroff et al. (1994). This technique ensured accurate assessment of cell population densities.

Measurement of Camphor Content

The camphor content quantification was accomplished through the utilization of a headspace gas chromatographic-mass spectrometer (GC-MS) system provided by Innovatech Labs, LLC, USA. This analytical setup was integrated with a Shimadzu QP2010 Ultra mass spectrometer and a Shimadzu AOC-5000 plus autosampler from Shimadzu Scientific Instruments, USA. The separation of compounds was achieved using a capillary column with a 30-meter length known as RTX-5M. The analysis commenced by introducing a camphor standard into the instrument. This enabled the determination of mass fragments and retention times associated with the camphor solution. To enhance the method's accuracy and precision, a selection was made of nine prominent ion peaks. A calibration curve was subsequently this data. Utilizing this curve, the camphor content within the samples was expressed in micrograms per gram (µg g^{-1}). generated employing During the GC-MS analysis, specific experimental parameters were maintained as follows: helium served as the carrier gas, an injection temperature of 250 °C was applied, an injection volume of 0.5 mL was employed, ionization voltage was set to 70 eV, a temperature of 100°C was sustained, and a heating duration of 10 minutes was observed. This comprehensive analytical approach allowed for the precise determination of camphor content, facilitating a comprehensive assessment of its presence within the *A. gypsicola* cell suspension cultures under diverse elicitation conditions and time points.

Statistical Analysis

The entire experimental procedures were conducted in triplicate to ensure robustness and reliability. The experimental design adopted a fully randomized layout. The collected data underwent thorough analysis through a 2-way analysis of variance (ANOVA) employing the Minitab 17 statistical software (Minitab, LLC, USA). To discern significant variations among means, the Tukey test was employed, and statistical significance was determined at a threshold of $p<0.05$. This analytical framework enabled the identification of noteworthy disparities and trends within the data.

Results and Discussion

Cell number, cell dry weight (g/L), and cell viability (%)

The variance analysis of cell number indicated significant differences ($p<0.01$) among the sorbitol treatment doses and sampling times (Figure 3). Accordingly, the application of sorbitol at concentrations of 5 and 25 g L^{-1} significantly increased cell numbers compared to the initial culture; however, these two doses exhibited a similar effect on cell numbers. The average cell count, initially at 82.900 cells, increased to 84.390 with the 5 g L^{-1} dose, but there was no further increase with the 25 g L^{-1} dose. On the other hand, the highest average cell count was achieved with the 50 g L^{-1} sorbitol treatment, reaching 90.010 cells. According to the relevant table, sampling times showed a significant effect on cell numbers. No statistically significant change in cell count was observed in samples taken on the first two days (84.865 and 85.350 cells, respectively), while a significant increase was seen in samples taken on the third day (86.060 cells).

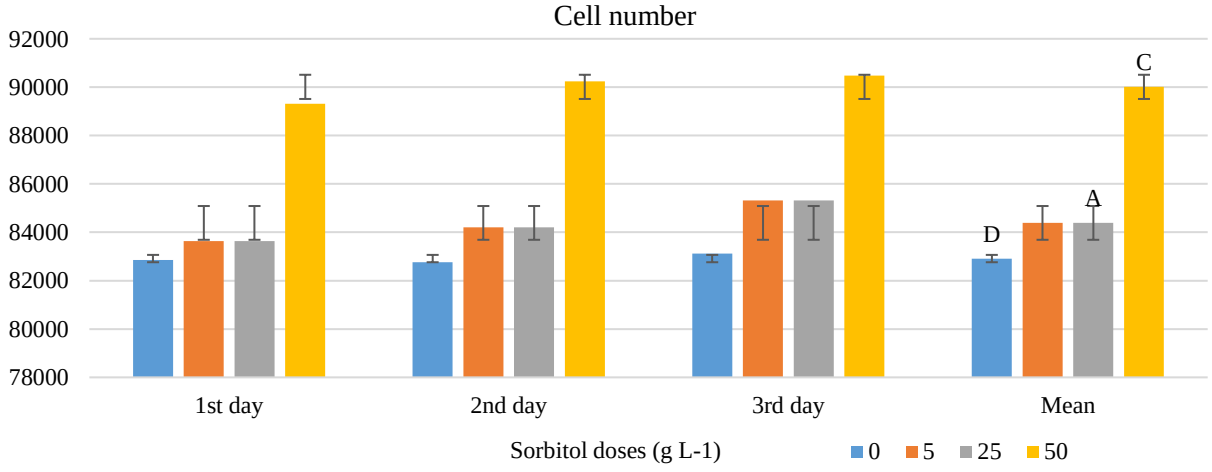

Figure 3. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on cell number in *A. gypsicola* cell suspension cultures

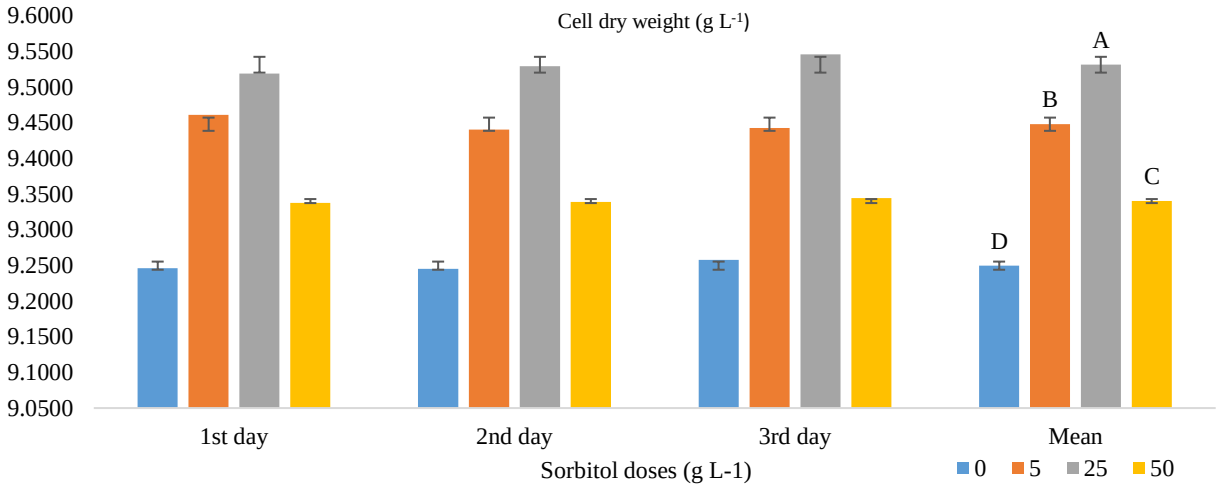

Figure 4. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on cell dry weight (g L^{-1}) in *A. gypsicola* cell suspension cultures

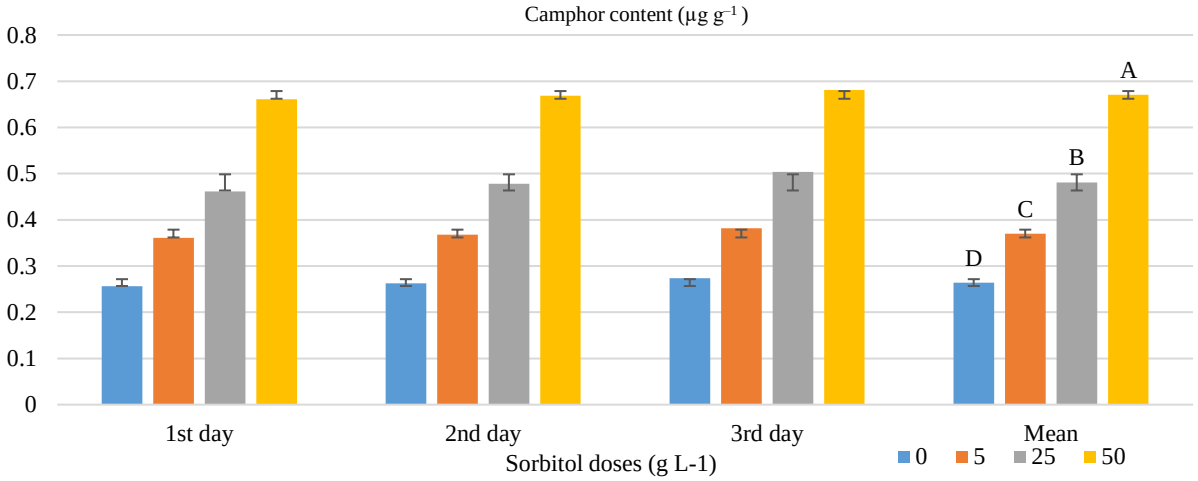

Figure 5. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on camphor content (µg g^{-1}) in *A. gypsicola* cell suspension cultures

For cell dry weight (g L^{-1}), variance analysis revealed significant differences between sorbitol doses ($p<0.01$) and sampling times ($p<0.05$). The initial culture's cell dry weight of 9.25 g L^{-1} increased to 9.34, 9.34, and 9.35 g L^{-1} with the doses of 5, 25, and 50 g L^{-1}, respectively. On the other hand, sampling times significantly influenced cell dry weight. The cell dry weight of 9.31 g L^{-1} in samples taken on the first day (8 hours after application) increased to 9.32 g L^{-1} on the second day and further to 9.32 g L^{-1} on the third day (Figure 4).

Regarding cell viability (%), variance analysis indicated significant differences ($p<0.05$) among sorbitol doses and sampling times. The corresponding descriptive statistical values and Tukey test results, demonstrated that at concentrations of 5 and 25 g L^{-1} of sorbitol, there was a significant increase in viable cell counts compared to the initial culture. However, both doses had a similar effect on the increase in viable cell count. In contrast, the application of 50 g L^{-1}sorbitol resulted in a decrease in the average viable cell count from 97.7% to 97.4% compared to the initial culture. Upon examining the relevant table, it is evident that sampling times had a significant effect on the average viable cell count. The viability, which was 97.6% on the first day, increased to 98.1% on the second day and then returned to 97.6% on the third day.

Numerous researchers have documented the substantial efficacy of elicitor treatments in enhancing cell growth and the production of secondary metabolites. Factors such as the developmental stage of the cell culture (Namdeo, 2007; Kang et al., 2009), duration of elicitor exposure, and the specific elicitor employed play pivotal roles in optimizing the outcomes of these treatments (Kubeš et al., 2014; Nazir et al., 2019; Açıkgöz, 2020a). Prior investigations have demonstrated that, akin to our findings, sorbitol treatment can significantly stimulate cell growth at appropriate concentrations (Al-Khayri and Al-Bahrany, 2002; Wu and Shi, 2008; de Costa et al., 2013; Zaker et al., 2015; Singh et al., 2017). Nonetheless, there exist studies in which sorbitol treatment has exhibited inhibitory effects on cell growth, leading to reductions in cell dry weight (Patil et al., 2013; Salehi et al., 2019; Ramulifho et al., 2019). Conversely, certain studies have emphasized the critical role of selecting suitable concentrations for maintaining cell viability during sorbitol treatments, highlighting that, as observed in this study, high concentrations of sorbitol may result in cell death (Hong et al., 2012; Valayil et al., 2015; Sarmadi et al., 2019).

Camphor Content (µg g^{-1}).

According to the variance analysis conducted for the camphor content (µg g^{-1}), while the difference between sorbitol doses was found to be significant ($p<0.01$), the variation among sampling times was deemed insignificant. The camphor content (µg g^{-1}) exhibited a significantly proportional increase with rising sorbitol doses (Figure 4). The initial culture's camphor content, which was 0.264 µg g^{-1}, increased by 40% to 0.370 µg g^{-1} at a dose of 5 g L^{-1}, by 82% to 0.4810 µg g^{-1} at a dose of 25 g L^{-1}, and by a remarkable 154% to 0.670 µg g^{-1} at a dose of 50 g L^{-1} (Figure 5). In cell cultures, sorbitol, which is commonly used to elevate osmolality, serves as a significant source of abiotic stress. It is well known that under stress conditions, cells increase their defense-oriented antioxidant enzyme levels, including CAT, POD, and SOD (Sytar et al., 2013; Vuleta et al., 2016; Azarabadi et al., 2017). Previous studies have demonstrated that sorbitol plays a crucial role in stimulating these enzymes and enhancing PAL activity within cells (Wu et al., 2008). Particularly, PAL and similar enzymes are the most active in the production of secondary metabolites within cells.

Numerous studies have reported that sorbitol elicitor promotes the accumulation of secondary metabolites and cell growth in cell cultures (Ling et al., 2008; Wu and Shi, 2008; Zhao et al., 2010; Patil et al., 2013; Valayıl et al., 2015; Zaker et al., 2015; Razavizadeh and Adabavazeh, 2017; Sing et al., 2017; Yang et al., 2017). Consistent with these findings, our own research has shown that the doses of sorbitol used in our study positively stimulate secondary metabolite accumulation and cell growth in cell cultures, underscoring its significance as an osmotic stress inducer.

Cells undergoing various growth stages in plant tissue culture systems exhibit distinct levels of mRNA and proteins, as indicated by Chong et al. (2005). This variance in cellular composition leads to differential responses to elicitor treatments, consequently influencing the accumulation of bioactive compounds, as demonstrated by Kang et al. (2009). As a result, precise calibration of the dosage and duration of elicitor treatments becomes crucial to effectively stimulate the generation of signaling molecules within the cells. Notably, specific concentrations of sorbitol employed in this investigation exhibited superior performance in enhancing the accumulation of camphor.

Conclusion

In this study, it has been determined for the first time that increasing doses of sorbitol applications in *A. gypsicola* cell suspension culture significantly enhance cell count, cell dry weight, and camphor content. The concentrations of sorbitol at 5 and 25 g L^{-1} were found to notably increase cell count compared to the initial culture, and cell dry weight increased in response to all three sorbitol doses. The initial camphor content of 0.264 µg g^{-1} increased by 40% to 0.370 µg g^{-1} at the dose of 5 g L^{-1}, by 82% to 0.481 µg g^{-1} at the dose of 25 g L^{-1}, and by an impressive 154% to 0.670 µg g^{-1} at the dose of 50 g L^{-1}. When evaluating cell count and cell dry weight based on sampling times, no statistically significant changes were observed in cell count for samples taken within the first two days, but a significant increase was seen in samples taken on the third day. On the other hand, sampling times were found to significantly affect cell dry weight. It was determined that cell dry weight increased in samples taken on the first, second, and third days. This research has demonstrated that the sorbitol doses used positively stimulate secondary metabolite accumulation and cell growth, thus highlighting its importance as a source of osmotic stress in cell cultures.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Effect of Potassium Humate on Soybean Germination Traits Under Salinity Stress Conditions

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ABSTRACT

Soybean is of strategic importance both as an oil crop and as a legume. Experiments have been conducted on soybean cultivation in saline soils. This study investigated the effects of salinity and K-humate concentrations on soybean germination. The findings contribute to our knowledge of soybean germination under salt stress and the potential use of potassium humate. The experiment was conducted in the laboratory of the Department of Field Crops at Adnan Menderes University, Türkiye. Seeds were surface-sterilized and placed on filter paper in Petri dishes. Different concentrations of water (control), NaCl solution (3 dS m^{-1}), and K-humate solution were added. According to the results, K-humate had a positive impact on germination rate. Significant differences were observed among control, salinity, salinity and K-humate applications. Salinity had an effect on germination and decreased the germination percentage but K-humate diminished the negative effects of salinity. These findings suggest the potential use of K-humate to enhance seedling establishment and overall plant productivity in salinity-affected environments.

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Introduction

Soybeans are one of the most significant crops worldwide due to their versatile applications in the food, feed, industrial, and health-related industries. Soybeans are a significant food source, but they are also utilized in a variety of industrial processes, such as the creation of biodiesel, plastics, and textiles. Because of their famed flexibility and plasticity, soybeans may be grown in a variety of settings and climates. As they can fix nitrogen in the soil and lessen the need for synthetic fertilizers, they are also a crucial crop for sustainable agriculture. Many nations, like the United States, Brazil, and Argentina, rely heavily on soybean exports, which are a key component of the world's food security (Fehr, WR 2007; Clemente and Cahoon,2009).

The majority of commercially important crops fall into the category of being moderately or highly susceptible to salinity. According to reports, crops have a 20–50% loss in yield as a result of salinity (Yamaguchi and Blumwald 2005). Salinity affects more than 33% of agricultural fields. According to current estimates, salinization is rising by 10% per year as a result of insufficient agricultural practices, saline irrigation water, climate change, high temperatures, and significant soil evaporation (Shrivastava and Kumar 2015). By 2050, it is predicted that salinity would damage 50% or more of agricultural fields (Jamil at all. 2011).

Osmotic stress and a lack of nitrogen delivery are problems for crops cultivated in salinity These elements severely harm plants by impairing growth, yield, photosynthesis, seed germination, and fruit quality (Murillo-Amador et al. 2007; El-Mogy et al. 2018). The early stage of growth known as the osmotic phase and the late stage of growth known as the ionic phase are when salt has an impact on plants (Munns and Tester 2008). The quantity of salt, the stage of growth, and the length of exposure all affect how plants react to it.

Soil salinity can affect germination in two ways: first, by creating an osmotic potential that prevents water uptake, and second, by the toxic effects of Na and Cl ions (Perez et al. 1998; Khajeh-Hosseini et al. 2003). According to Almansouri et al. (2001), salinity is a significant factor that either prevents or delays seed germination and seedling establishment. Researchers Munns (1993) and Bajehbaj (2010) found detrimental effects of salinity on seed germination.

Through soil (dos Santos et al. 2021) and foliar (Abdelrasheed et al. 2021) treatments, potassium humate (Kh), a salt made from humic acid (HA), is used to enhance plant growth and output. By enhancing the cell membrane's permeability, HA is thought to facilitate the passage of nutrients from the soil into the plant, hence promoting plant growth and yield (Noroozisharaf and Kaviani 2018). Additionally, it has been noted that HA promotes antioxidant enzyme activity and enhances plant growth and photosynthesis under abiotic stressors (Kaya et al. 2018). Furthermore, potassium (K) is categorized as a macroelement that is necessary for the majority of physiological activities in plants (Mridha et al. 2021). Potassium has a beneficial effect on reducing stressors such salt and drought (Kumari et al. 2021). Potassium humate increases the rate of nutrient uptake, enhances plant biomass and reduces the soil compaction (Canellas et al., 2015). Previous research has shown that HA can help crops like sorghum cope with salt stress (Ali et al. 2019). The impact of exogenous potassium humate treatment on beans has been the subject of a few research, such the one by Taha and Osman (2018), who discovered that potassium humate enhances bean development under salt stress.

This study was conducted to investigate the effects of different salt and potassium humate concentrations on germination parameters of soybeans. The findings of this study will contribute to our knowledge of soybean germination under salt stress conditions and provide insights into the potential use of potassium humate as a mitigating agent.

Material and Methods

This experiment was carried out in the laboratory of Department of Field Crops in Adnan Menderes University University, Türkiye.

The seeds of soybean were surface-sterilized with 3% Formaldehyde for 10 minutes and washed 3 times with re-distilled water. Twenty five seeds were placed Whatmann filter-paper in per Petri dish with 9 cm diameter and added 7 mL of either water (control) or NaCl solution (3 dS $m^{-1)}$ and K-humate solution (Figure 1). Research design was based on two factor-factorial randomized parcels with four replicates. The first factor was salinity; a control and 3 dS/m were used. The second factor was K-humate was used as a control, and concentration of Kh was 0.3 g L^{-1} were used.

Every day at a specific time, seed germination was recorded. When a seed's radicle had grown to a length of around 2 mm, it was considered to have germinated (Mohammadi, 2009). Radicle and plumule length were measured after 7 days. After that, seedlings were dried in an oven for 24 hours at 105°C, and the scale's precision was set to 0.001 (ISTA, 2003).

In this trial, the following agronomic characteristics were examined: fresh cotyledon weight, dry cotyledon weight, dry weight of hypocotyl, fresh weight of hypocotyl, length of hypocotyl, dry weight of roots, fresh weight of roots, root length, germination rate, germination vigor, and relative water content (RWC).

$$RWC = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight})]} \times 100$$

Root and cotyledon plant samples were dried at 65 °C, weighed, and ground. Utilizing a microwave digestion system, concentrated HNO3-H2O2 (6:2, v/v) nitric acid (65% Merck) was used to break down dried plants. After cooling, the resultant solutions were diluted in volumetric flasks with distilled water to a maximum concentration of 50 mL. By using ICP-OES Agilent 5800 VDV, an inductively coupled plasma atomic emission spectrometer, element contents (K, Na, and P) of the final solution were determined.

Results and Discussion

In this study, character of germination rate, fresh weight of hypocotyl, dry weight of hypocotyl, length of hypocotyl, fresh weight of roots, root length, dry weight of roots, length of roots, fresh cotyledon weight, and relative water content affected significantly by salinity and length of hypocotyl affected signaficantly by K- humate. A statistically significant interaction was observed between salinity and K-humate treatments regarding the fresh weight of hypocotyl, dry weight of hypocotyl, and fresh weight of roots (Table 1).

As expected, salinity stress (3 dS/m^{-1}) reduced the germination percentage, fresh herba weight, dry herba weight, root length, fresh cotyledon weight, and relative water content of soybean plants. However, under k-humate treatment, all germination and morphological parameters increased compared to the control treatment. When comparing k-humate and salin conditions to control plants, germination rate, fresh root weight, and relative water content were in the same statistical group, while germination percentage, fresh herba weight, dry herba weight, hypocotyl length, dry root weight, root length, and fresh cotyledon weight were lower. Furthermore, there was no significant difference in the dry cotyledon weight among all treatments (Table 2).

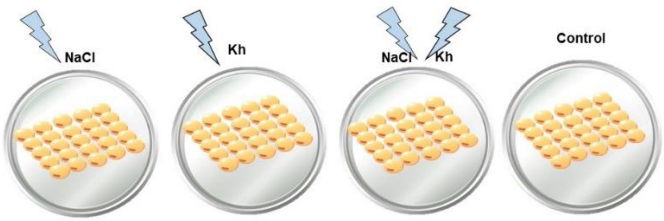

Figure 1. Scheme of the experimental treatments

Table 1. Analysis of variance for the effect of different treatments on some morphological and germination parameters of soybean seeds.

		Mean Square					
Source of Variance	df	GR	GP	FHW	DHW	FRW	DRW
Salinity (S)	1	505.01*	163.84*	0.413*	0.014**	0.072*	0.006**
K- Humade (K)	1	0.02	42.90	0.052	0.002	0.033	0.001
S and K	1	301.89*	71.4	0.233*	0.014**	0.104**	0.000
Error	12	55.311	33.35	0.047	0	0.012	0
		Mean Square					
Source of Variance	df	RL	FCW	DCW	RWC	HL	
Salinity (S)	1	1.898**	10.240*	0.006	1.040*	1.630**	
K- Humade (K)	1	0.334	4.818	0.007	0.129	1.247**	
S and K	1	0.005	0.026	0.000	0.017	0.165	
Error	12	0.085	1.33	0.015	0.160	0.042	

GR: Germination rate, GP: Germination percentege, FHW: Fresh weight of hypocotyl, DHW: Dry weight of hypocotyl, FRW: Fresh weight of roots, DRW: Dry weight of roots, RL: Length of roots, FCW: fresh cotyledon weight, DCW:Dry cotyledon weight, RWC: Relative water content, HL:length of hypocotyl.

Table 2. Means of different treatments on some morphological and germination parameters of soybean seeds

Treatments	GR	GP	FHW	DHW	HL	FRW	DRW	RL	FCW	DCW	RWC
CONTROL	50.75 AB	82.50 A	0.98 B	0.15 AB	1.79 B	0.55 B	0.11 A	2.57 A	7.70 A	1.56	2.06 A
SAL	49.38 AB	75.62 B	0.90 B	0.15 AB	1.36 C	0.57 B	0.07 B	1.84 B	6.02 B	1.51	1.61 B
Kh	59.38 A	81.55 A	1.33 A	0.23 A	2.55 A	0.80 A	0.13 A	2.82 A	8.72 A	1.59	2.30 A
SAL × Kh	40.63 AB	79.37 B	0.77 C	0.11 C	1.71 C	0.50 B	0.09 B	2.17 B	7.20 B	1.56	1.73 A

+ Different letters in rows indicate differences among treatments according to LSD test ($p < 0.05$). GR: Germination rate, GP: Germination percentege, FHW: Fresh weight of hypocotyl, DHW: Dry weight of hypocotyl, FRW: Fresh weight of roots, DRW: Dry weight of roots, RL: Length of roots, FCW: fresh cotyledon weight, DCW: Dry cotyledon weight, RWC: Relative water content, HL: length of hypocotyl.

Based on the ANOM-decision charts (Figure 2), when examining the average %K content, it was found that the root had an average of 4.08, while the cotyledon had an average of 2.11. In both plant parts, the highest average was observed in the k-humate application. The average %Na content was measured as 0.44 in the root and 0.19 in the cotyledon, indicating a higher accumulation of Na in the root compared to the cotyledon. When comparing the plant parts, values above the average were obtained in both k-humate and salinity*k-humate applications. In terms of %P values, the root average (0.54) was higher than the cotyledon average (0.48). Looking at the P content in the root, values below the average were observed in the control group, while all other treatments had values above the average. In the cotyledon, below-average P accumulation was detected in the control and salinity*k-humate applications.

Salt stress reduces plant development by altering the activity of the hormones and enzymes involved in photosynthesis (Nasrallah et al. 2022) Our study shows that salinity decreased the growth of soybean seedlings. This situation can be attributed to a multitude of factors, including the ionic effect, osmotic pressure, limitations in the uptake of essential elements, a reduction in the photosynthetic rate, and Na concentrations in plant tissues (Abdeldym et al. 2020; Abbas et al. 2022). This study, Kh an salinity*kh applications increased plant germination parameters under normal and salininity conditions.

According to earlier studies that concur with our findings, Kh plays a beneficial impact in promoting soybean plant growth under both normal and salinity stress (Taha and Osman, 2018; Hemida et al. 2017, Mahdi et al. 2021). According to Hemida et al. (2017), Mahdi et al. (2021), Osman and Rady (2012), the beneficial effects of Kh on plant growth may be attributed to its capacity to increase the organic matter in growth media, make more water available, prevent mineral nutrients from leaching, and increase mineral absorption by plant roots. Additionally, this might be as a result of role of potassium in regulating various plant enzymes (Kumar et al. 2020) and humate's function as a biostimulant (Shalaby et al. 2023). Our results indicate that the most effective treatment is KH application and this result supports findings of Shalaby et al. (2023).

Salinity influences the uptake and accumulation of elements in plant tissue (Munns and Tester, 2008). In the rhizosphere, it is well known that excessive levels of Na and Cl have negative effects on the uptake of nutrients (N, P, K^+, Ca, and Mg, as well as microelements) (Abdeldym et al. 2020).

Salinity decreased the level of Na+ and raised the amount of K+ in the root and cotyledons in this investigation in Figure 2. According to a different set of findings by Saidimoradi et al. (2019), strawberry plants' K+ absorption was decreased by salinity stress.

Negative salinity conditions could be mitigated by maintaining enough K^+ levels in plants (Nadeem et al. 2019; Naeem et al. 2020; Farag et al. 2022) our results show that Na^+ content in leaves was increased in Kh and Kh * Salinity treatments (Figure 2).

Additionally, K^+ is a component in Kh, and it is known that K^+ increases salinity resistance because it competes with sodium to bind and regulate the water status of plants (Capula-Rodríguez et al. 2016). The adsorption of Na by humic substances as a result of Kh treatment aids in the reduction of Na content in common bean shoots and allows the roots to absorb more K+ (Lakhdar et al. 2009).

Salinity is the cause of osmotic stress in plants, and relative water content (RWC) in leaves is a good indicator of how well-tolerated osmotic stress is by a plant. In line with the findings of Hasanuzzaman and Fujita (2013), the current investigation found that saline stress greatly reduces the RWC content (Table 2).

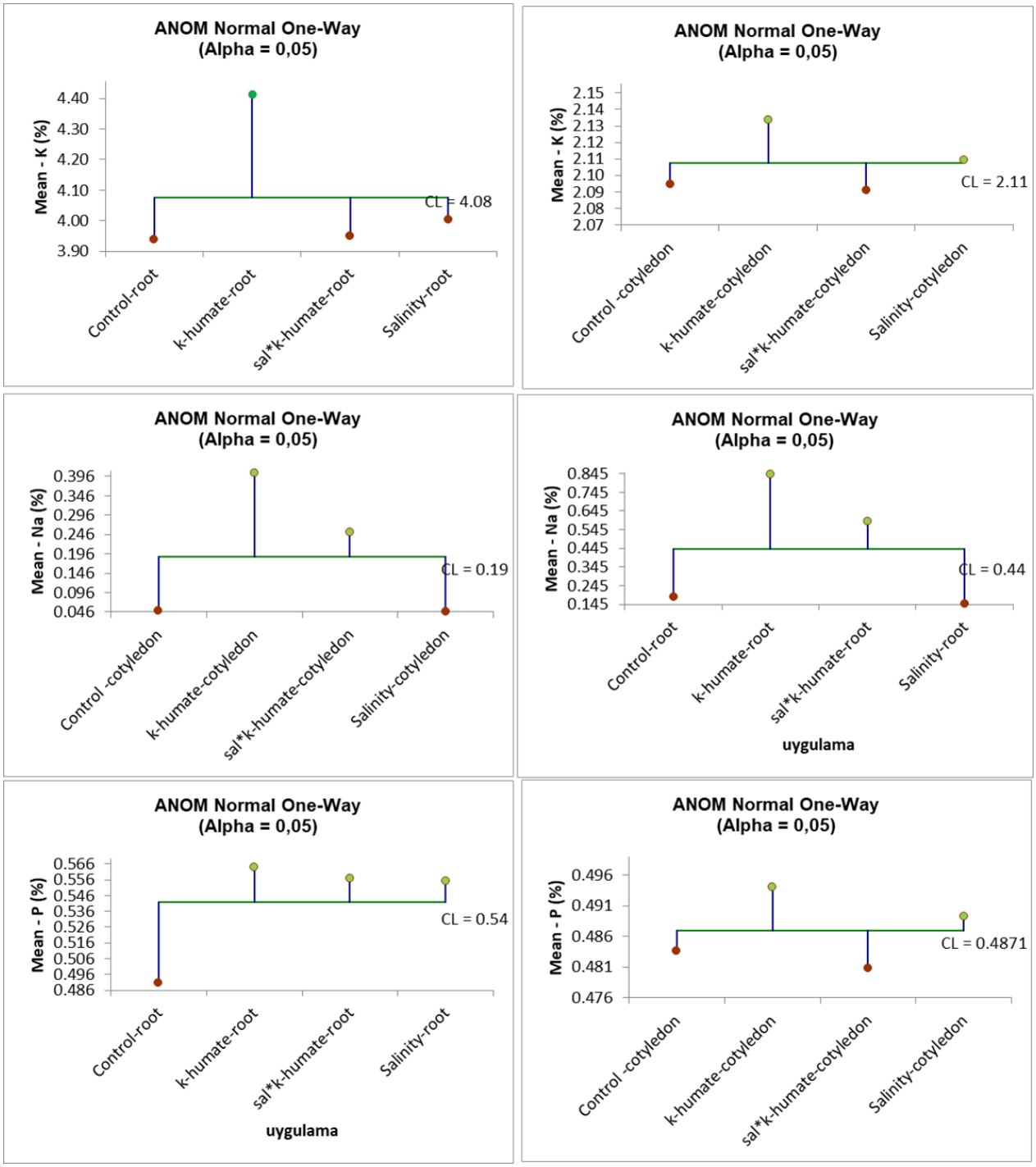

Figure 2. ANOM-decision chart with average means plant nutritients (K, Na, P, %) across root and andcotyledon (alpha > 0.05%) .Red-coloured heads represent significant deviation lower decision level and green-coloured above upper decision level from average.

(From "The Analysis of Means: A Graphical Method for Comparing Means, Rates, and Proportions," by Peter R. Nelson, Peter S. Wludyka and Karen A. F. Copeland)

However, the application of K-humate enhanced the RWC of leaves under saline conditions to a considerable extent because Kh*salinity helps to retain turgidity by reducing water loss under stress conditions (Hasanuzzaman et al., 2018).

Conclusion

Based on the results of this study, it appears that K-humate has a positive impact on germination rate. Statistically significant differences were observed among the control, salinity, and salinity * K-humate applications. When examining the FHW attribute, the K-humate application was found to have the highest positive effect compared to other treatments. The germination percentage, on the other hand, was observed to be reduced by salinity. The salinity conditions statistically decreased the germination percentage value. In terms of % Na (cotyledon), the highest measurement was recorded in the K-humate application, followed by the K-humate * salinity application. In the presence of K-humate, the negative impact of salinity on germination was mitigated, resulting

in improved germination performance. These findings highlight the potential of K-humate as a beneficial factor in promoting germination under salinity stress conditions. The observed increase in germination rate suggests that K-humate application could be a promising strategy to enhance seedling establishment and overall plant productivity, particularly in salinity-affected agricultural environments.

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Clinical Effect of Poly Herbal Unani Formulation on Dyslipidemia- A Randomized Trial

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ABSTRACT

Background: In adults aged 30-70 with primary and moderate hyperlipidemia, the present study took place to investigate the therapeutic benefits of a polyherbal unani preparation called Garlitab. *Methods***:** It was a prospective open label, herbal coded test drug-controlled, randomized trial. Out of total screened patients we were enrolled 212 hyperlipidemic patients of 30–70 years in the study fulfilling the inclusion criteria, we were recruited them from OPD of a hospital in Munshiganj and different Unani clinics of Dhaka, Bangladesh after obtaining written informed consent from the patients. Selected individuals were allocated into two groups at random. Group1 Received 500 mg Garlitab tablets twice daily and Group 2 received tablet atorvastatin calcium 10 mg 2 times daily. Height, weight, and blood pressure were recorded along with blood samples. The random distributions were carried out by a research assistant utilizing a random numbers table. Blood samples were taken at the beginning of the trial, 1.5 months later, and 3 months following the intervention. *Results:* Results for the test medication revealed a substantial drop in cholesterol levels between baseline and the data collected after three months and in case of male it was from 241.72±38.11 to 218.24±34.06 mg/dL for total cholesterol, from 198.27±30.57 to 173.54±29.34 mg/dL for LDL and from 280.78±85.81 to 207.07±51.40 mg/dL for triglyceride. HDL increases from 33.05±3.21 to 34.69±3.13 mg/dL in male patients. The control drug atorvastatin calcium also showed a significant decrease in lipids between baseline and after 3 months data and in case of male it was from 241.92±31.54 to 174.90±22.87 mg/dL for total cholesterol, from 196.20±30.91 to 130.30±24.29 mg/dL for LDL and from 279.48±115.35 to 141.27±59.55 mg/dL for triglyceride. It increases HDL from 32.00±2.25 to 34.03±2.19 mg/dL in male patients. Between the baseline and the 3-month data, the test medicine for females significantly reduced total cholesterol, LDL, and triglycerides and it was from 244.64±52.18 to 220.12±45.07 mg/dL, from 200.32±30.57 to 173.54±29.34 mg/dL and from 272.32±99.69 to 195.25±60.68 mg/dL respectively. HDL increases from 33.77±3.36 to 35.03±3.23 mg/dL. Between the baseline and the 3-month data, the control medication for females significantly reduced total cholesterol, LDL, and triglycerides and it was from 247.74±37.95 to 175.26±29.54 mg/dL, from 197.65±27.89 to 130.91±22.04 mg/dL and from 271.57±94.52 to 142.00±50.88 mg/dL respectively. It increases HDL from 32.22±2.32 to 33.46±2.94 mg/dL. *Conclusions:* According to the results of the study, the polyherbal formulation Garlitab can lower cholesterol levels. It may be a useful medication for treating primary hyperlipidemia.

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Introduction

Dyslipidaemias involve modifications to the plasma lipid profile that often correspond to clinical disorders. Nevertheless, some kinds of dyslipidaemia, such as hypertriglyceridaemia, are linked to serious disorders in other organ systems, such as non-alcoholic fatty liver disease and acute pancreatic inflammation. Dyslipidaemias, Major risk factors for heart attack and stroke involve excessive plasma LDL-C (Low-Density Lipoprotein) holesterol amounts in particular. Dyslipidaemias can develop as a result of secondary conditions, with the second category occurring more frequently, such as diabetes mellitus, obesity, or an unhealthy lifestyle. As a result of primary or familial dyslipidaemias, genes may also be to blame. In 1990, increased plasma LDL-C (Low-Density Lipoprotein) holesterol ranked as the 15th most important risk factor for death. By 2007 and 2019, it has risen to the 11th and 8th positions, respectively. Because of the condition's elevated risk of heart attacks and strokes, it is the most common form of dyslipidaemia (Pirillo et al., 2021). Dyslipidaemia, a name used to refer to variations in lipids, comprises high levels of triglycerides (TG), low levels of HDL-C (High-

Density Lipoprotein), a rise in levels of LDL-C (Low-Density Lipoprotein), and elevated amounts of total cholesterol (TC) (Mohamed-Yassin et al., 2021). One of the known risk factors for cardiovascular disease is dyslipidemia. (Mohamed-Yassin et al., 2021). Comprehensive evaluations reveal a substantial relationship between high LDL-C (Low-Density Lipoprotein) and atherosclerotic cardiovascular disease (Cardiovascular Disease (CVD)) (Legrand et al. 2013; Collins et al., 2016). Additionally linked to Cardiovascular Disease (CVD) was low HDL-C (High-Density Lipoprotein) (Di Angelantonioet al., 2009). The development of atherosclerosis and Cardiovascular Disease (CVD) has been associated with high TC and LDL-C (Low-Density Lipoprotein), although more recent research has cast doubt on this relationship (Stückle, 2015; Ravnskov et al., 2018).

The impact of boosting HDL-C (High-Density Lipoprotein) in preventing Cardiovascular Disease (CVD) has also been questioned, even though some studies have shown that non-HDL-C (High-Density Lipoprotein) predicts CV risk better than LDL-C (Low-Density Lipoprotein) (Robinson et al., 2009; Arsenault et al., 2009).

In comparison to Europe (53.7%) and America (47.7%), South East Asia (30.3%) and Africa (23.1%) had much lower prevalence rates of hypercholesterolaemia in adults. (Mohamed-Yassin et al., 2021). The prevalence rates reported by Lin et al., (Lin et al., 2016) Nevertheless, ranged widely throughout the Asia-Pacific countries, from 9% in Indonesia to 46.9% in the Philippines. High TG, low HDL-C (High-Density Lipoprotein), and high LDL-C (Low-Density Lipoprotein) are more common, with prevalence rates ranging from 7.8% to 47.2%, 13.9% to 38.6%, and 10.1% to 71.3%, respectively (Lin et al., 2016). In Noubiap et al.'s 2018 comprehensive analysis of dyslipidemia in Africa, the prevalence of increased TC, high LDL-C (Low-Density Lipoprotein), elevated TG, and low HDL-C (High-Density Lipoprotein) was reported to be 25.5%, 28.6%, 17%, and 37.4%, respectively (Noubiap et al., 2015).

Due to rising urbanization, socioeconomic growth, longer life expectancies, insufficient nutrition, and alterations in lifestyle, the population of Southeast Asia is currently at an increased risk of Cardiovascular Disease (CVD) (Choudhury et al., 2014). One of the developing countries in Southeast Asia, Bangladesh, has seen an increase in the incidence of non-communicable diseases and accompanying mortality during the previous several decades (Saquib et al., 2012). A recent examination of the literature found that adult Bangladeshis living in urban regions are more likely to have Cardiovascular Disease (CVD) than those residing in remote regions (Chowdhury et al., 2018). Several studies support this (Islam et al., 2012; Mithal et al., 2014; Joshi et al., 2014), In Bangladesh and other South Asian nations, dyslipidemia—a modifiable risk factor for Cardiovascular Disease (CVD)—is on the rise.

Our study drug Garlitab is a polyherbal Unani composition made of six priceless herbs, including Garlic *(Allium sativum)* dry extract, Onion *(Allium cepa)* dry extract, Black plum *(Syzygium cumini)* seed dry extract, Mango *(Mangifera* indica) leaf dry extract, Nutmeg (Myristica fragrans) fruit dry extract, Clove (Syzygium aromaticum) flower dry extract. It is designed to treat hyperlipidemia, particularly by reducing the LDL-C (Low-Density Lipoprotein) level of blood.

Clove (Syzygium aromaticum)

The spice name clove refers to the tiny, dried flower buds of Eugenia caryophyllata, also known as *Syzygium aromaticum*, which are reddish brown in color (Habtemariam et al., 2019).). Figure 1, 2 and 3 is the picture of: Dried cloves, Clove tree flower buds and the compound eugenol is responsible for most of the characteristic aroma of cloves.

Figure 1. Dried cloves. (Source- Internet)

Figure 2. Clove tree flower buds. (Source- Internet)

Clove (Syzygium aromaticum) is being used to treat dyslipidaemias for long time. According to one clinical study published in 2017, Clove supplementation resulted in statistically significant increases in HDL cholesterol and the highest reductions in total cholesterol, triglycerides, LDL, and VLDL cholesterol in the hyperlipidemic group (Balasasirekha et al., 2012).

Garlic (Allium sativum)

Garlic (Allium sativum) is a kind of bulbous plant with flowers that belonging to the Allium genus. Amongst its nearest cousins are the onion, shallot, leek, and chive (Block et al., 2010). Numerous civilizations, especially Egypt, Japan, China, Rome, and Greece, utilized garlic as a traditional alternative (Garlic, 2022). Figure 4, 5 and 6 is the picture of: Raw Garlic, Garlic Plants and Alliin Garlic contains the sulfur-containing substance

Garlic is traditionally used for hyperlipidemic patients. A parallel-designed randomized controlled clinical study included 112 hyperlipidemic individuals between the ages of 30 and 60. After receiving garlic and lemon juice, those with hyperlipidemia showed improvements in the level of blood pressure, fibrinogen levels, and cholesterol (Aslani et al., 2016).

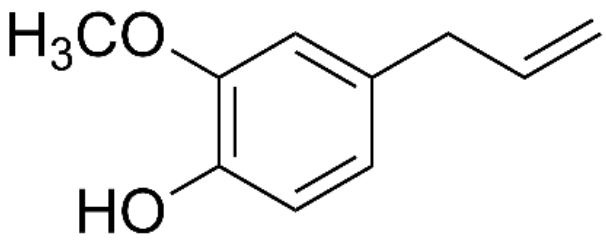

Figure 3. The compound eugenol is responsible for most of the characteristic aroma of cloves. (Source- Internet)

Figure 4. Raw Garlic. (Source- Internet)

Figure 5. Garlic Plants. (Source- Internet)

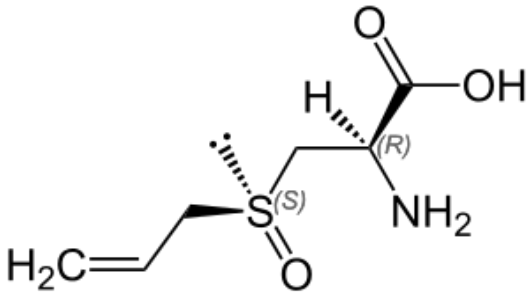

Figure 6. Garlic contains the sulfur-containing substance alliin. (Source- Internet)

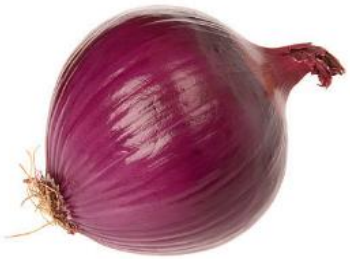

Figure 7. Raw Onion. (Source- Internet)

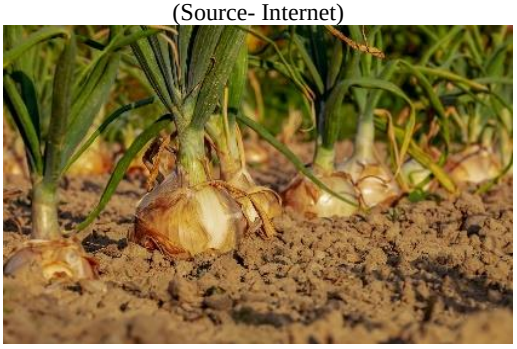

Figure 8. Onion Plants. (Source- Internet)

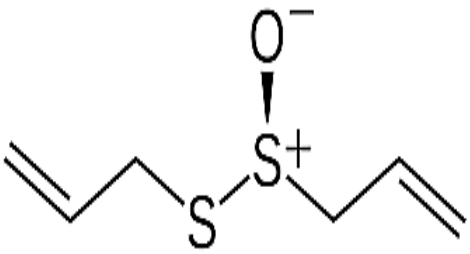

Figure 9. Onion contains the sulfur-containing substance allicin. (Source- Internet)

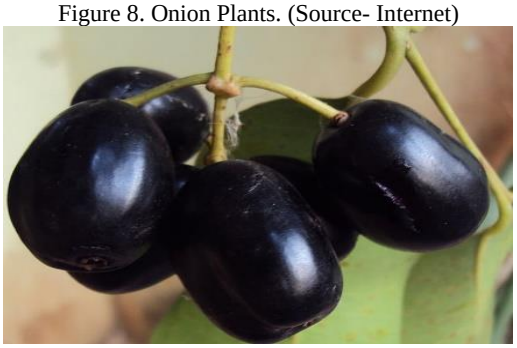

Figure 10. Raw Black plum. (Source- Internet)

Figure 11. Black plum Seed. (Source- Internet)

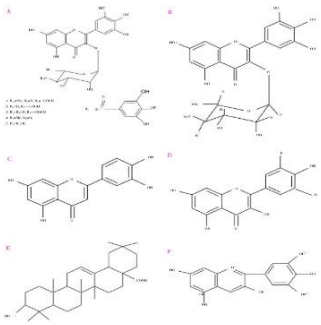

Figure 12. (1) Mearnsetin, Myricetin, (2), Myricetin-3-O-(400-O-acetyl)-a-L-rhamnopyranoside (3), Myricet Gentiobiose, (Source- Internet)

Onion (Allium cepa)

The most commonly farmed a member of the genus Allium is the onion (Allium cepa L., from the Latin cepa, meaning "onion"). It is additionally frequently referred to as the bulb onion or the common onion (Khoury et al., 2013). Numerous health advantages, including a decrease in LDL cholesterol, blood pressure reduction, have been linked to allicin found in studies (Medicalnewstoday.com, 2022). Figure 7, 8 and 9 is the picture of: Raw Onion, Onion Plants and Allicin Onion contains the sulfur-containing substance

Black plum (Syzygium cumini)

Known for its berries, timber, and ornamental value, Syzygium cumini is an evergreen tropical tree in the Myrtaceae flowering plant family. Additionally, it goes by the names jamun, jaman, jambul, jambon, Malabar plum, Java plum, black plum, etcetera. Figure-10, 11 and 12 is the picture of: Raw Black plum, Black plum Seed and (1) Mearnsetin, Myricetin, (2), Myricetin-3-O-(400-O-acetyl)-a-L-rhamnopyranoside (3), Myricet Gentiobiose.

The jambolan has a long history in complementary medicine, and all the components of the plant can be used medicinally (Reynertson et al., 2005). Syzygium cumini seed powder administration substantially decreased white adipose tissue (WAT) weights, blood sugar, serum insulin and plasma lipids including total cholesterol, triglyceride, LDL, and HDL content (Sharma et al., 2017).

Mango (Mangifera indica) leaf

For thousands of years, the leaves of a certain type of mango, *Mangifera indica*, have been utilized in traditional Chinese medicine, Unani medicine, and the medicine of Ayurveda (Batool et al., 2018). Figure-13, 14 and 15 is the picture of: Raw Mango leaf, Mango tree, Chemical make-up of a few phytochemicals present in mango fruit and plants.

Mangifera indica leaf extract, which is high in phytosterols, is a great source of a nutraceutical element that may decrease blood cholesterol levels. Substantial cholesterol-lowering effects were shown in rats given a methanol extract of M. indica leaves at a dose of 90 mg/kg body weight, and a dose of 5000 mg/kg rat body was also demonstrated to be harmless (Gururaja, 2017).

Figure 13. Raw Mango leaf. (Source- Internet)

Figure 14. Mango tree. (Source- Internet)

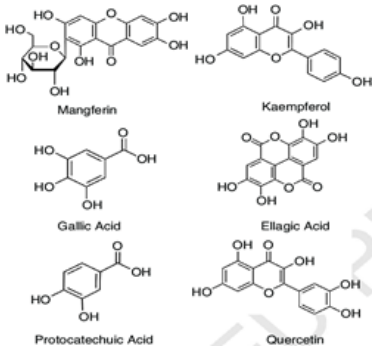

Figure 15. Chemical make-up of a few phytochemicals present in mango fruit and plants (Kabir, Y et al.,2017).

Figure 16. Dried Nutmeg. (Source- Internet)

Figure 17. Nutmeg tree. (Source- Internet)

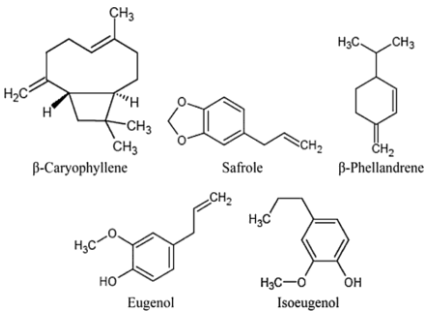

Figure 18. The chemical make-up of several significant nutmeg seed oil elements includes caryophyllene, safrole, phenanthrene, eugenol, and isoeugenol (Gupta E, 2020).

Nutmeg (Myristica fragrans)

An evergreen tree with dark-green leaves known as the fragrant nutmeg or real paprika (M. fragrans) is planted for two spices that are prepared from its fruit. The seed from several Myristica tree species, or the ground spice made from that seed, is known as nutmeg (FAO, 2018). Figure-16, 17 and 18 is the picture of: Dried Nutmeg, Nutmeg tree and the chemical make-up of several significant nutmeg seed oil elements includes caryophyllene, safrole, phenanthrene, eugenol, and isoeugenol.

Methods

Type of study

With a 1:3 allocation ratio to the intervention and herbal-coded test drug groups, this study was a prospectively open-label, herbal-coded test drug-controlled, randomized analysis.

Study population

The study included participants of both sexes with abnormal lipid profiles who were 30 to 70 years old and receiving therapy for primary hyperlipidemia.

Sample size

Random sampling was used to choose and register patients. Following screening tests such a fasting lipid profile, an average of 2–3 responders were registered each clinic day. The clinical trial had 212 participants in total. After satisfying both the inclusion and exclusion requirements, the test and positive control groups each included 3:1 patients (test n=159, positive control n=53). Figure 19 is the RCT Flow Diagram.

Sampling technique

Subjects were randomly assigned to receive either the herbal product or the intervention. Each supplement has a code correlating to the trade name box. Participants and the researcher in charge of participant recruitment, data collection, and analysis (MM) were aware of the supplements each participant received.

Inclusion criteria

Both male and female participants is included if they-give their consent to participate the study, Age 30–70 years, a fasting LDL cholesterol reading that is higher than159 mg/dL.

Exclusion criteria

Any gastrointestinal conditions that could interfere with the intestinal activities and absorption of the polyphenols were grounds for exclusion from the study; was taking any drugs that might have affected outcomes, such as those for controlling blood pressure, blood sugar, or cholesterol; was using additional natural health supplements, such as fish oil or phytosterols, which are known to affect cholesterol or polyphenols; a significant health condition (such as liver/thyroid problems or a recent major surgery); are nursing or pregnant; smoked cigarettes or had a cardiac defibrillator installed. If a participant had a history of, or is now experiencing, depression, anxiety, or indicators of cognitive deterioration, they were disqualified from the cognitive and mood tests.

Data collection

In addition to baseline biochemical measurements, fasting (>11 hours) venous blood samples were also collected at 1.5 and 3 months.

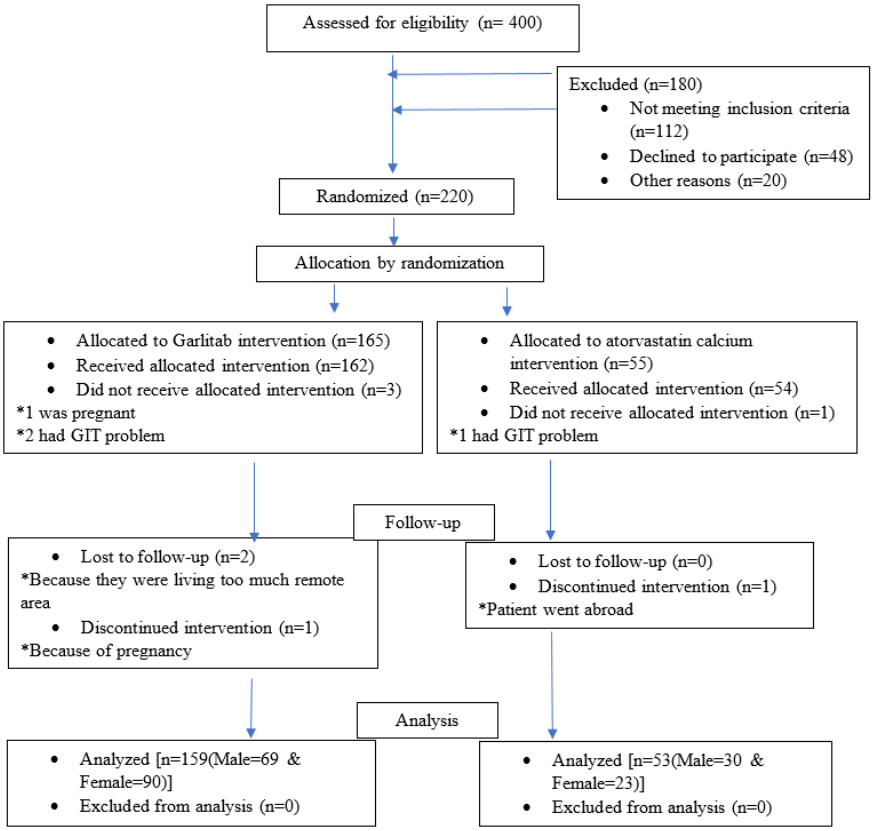

Figure 19. RCT Flow Diagram

Data analysis

Mean and standard deviation were calculated along with descriptive statistics. Cross tabulation and chi square statistics were used to identify associations between socio-demographic factors. SPSS (version 18, SPSS, Chicago, IL, USA) was used to analyze the data, and a two-sided P value of 0.05 was regarded as significant. Blood samples were taken at the start of the trial to create a baseline, after 1.5 months of intervention, and after 3 months of intervention in order to quantify parameters of metabolic profiles. To assess the effectiveness of baseline data and data after three months of lipids, a two-tail paired t-test was performed for the main effect study. Chi-square analysis for categorical data was used to analyze baseline general features. When we got a significant result in the two-tail paired t-test, we performed Tukey's post hoc comparisons to determine pairwise differences.

Results

Response on total cholesterol levels of test group

159 individuals received the test medication, 69 of them were men and 90 of whom were women (Table 1A, B). The baseline mean total cholesterol level for male responders was 241.72±38.11 mg/dL. According to Table 10, total cholesterol levels decreased after 6 weeks of medication treatment to 227.20±36.47 mg/dL and after 12 weeks to 218.24±34.06 mg/dL. For female responders, the mean total cholesterol level was 244.64±52.18 mg/dL at baseline. As indicated in table 11, total cholesterol levels decreased after 6 weeks of medication delivery to 228.24±48.10 mg/dL and after 12 weeks to 220.12±45.07 mg/dL.

Table 1(A). Age distribution by treatment group according to gender.

	Age group	Treatment group			
		Test group		Positive Control group	
		N	(%)	N	(%)
Male	≤35 years	15	21.7	11	36.7
	36-45 years	24	34.8	8	26.7
	46-55 years	17	24.6	6	20.0
	56-65 years	12	17.4	4	13.3
	>65 years	1	1.4	1	3.3
	Total	69	100.0	30	100.0
Female	≤35 years	22	24.4	6	26.1
	36-45 years	39	43.3	12	52.2
	46-55 years	19	21.1	3	13.0
	56-65 years	9	10.0	2	8.7
	>65 years	1	1.1	0.0	0.0
	Total	90	100.0	23	100.0

Table 1(B). Age distribution of the treatment group (Average).

Age group	Test group		Positive Control group	
	N	(%)	N	(%)
≤35 years	37	23.3	17	32.1
36-45 years	63	39.6	20	37.7
46-55 years	36	22.6	9	17.0
56-65 years	21	13.2	6	11.3
>65 years	2	1.3	1	1.9
Total	159	100.0	53	100.0

Table 2. Mean age and SD distribution of treatment group according to gender.

Treatment group	Sex	Mean	Number	Std. deviation
Positive control group	Male	44.07	30	11.350
	Female	41.91	23	8.570
	Total	43.13	53	10.202
Test group	Male	45.38	69	10.586
	Female	43.48	90	9.401
	Total	44.30	159	9.945
Total	Male	44.98	99	10.782
	Female	43.16	113	9.223
	Total	44.01	212	9.999

Table 3. Distribution of treatment group according to gender.

Sex	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Male	69	43.4	30	56.6
Female	90	56.6	23	43.4
Total	159	100.0	53	100.0

Table 4. Distribution of treatment group according to Occupation.

Occupation	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Unemployed	11	6.9	2	3.8
Service holder	42	26.4	21	39.6
Agricultural work	13	8.2	1	1.9
Business	11	6.9	6	11.3
Day laborer	3	1.9	-	-
House wife	72	45.3	17	32.1
Retired	7	4.4	6	11.3
Total	159	100.0	53	100.0

Table 5. Distribution of treatment group according to Diabetes.

Diabetes	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Type 1 DM	25	15.7	5	9.4
Type 2 DM	4	2.5	5	9.4
No diabetes	130	81.8	43	81.1
Total	159	100.0	53	100.0

Table 6. Distribution of treatment group according to treated hypertension

Treated HTN	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Yes	34	21.4	11	20.8
No	125	78.6	42	79.2
Total	159	100.0	53	100.0

Table 7. Distribution of treatment group according to smoking history

Smoking history	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Current smoker	42	26.4	22	41.5
Non smoker	117	73.6	31	58.5
Total	159	100.0	53	100.0

Table 8. Distribution of treatment group according to Body Mass Index

BMI classification (Asian)	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Under weight <18.5	-	-	-	-
Normal range 18.5-22.9	59	37.1	28	52.8
Over weight 23.0-27.5	97	61.0	25	47.2
Obese c ≥ 27.5	3	1.9	-	-
Total	159	100.0	53	100.0

Table 9(A). Distribution of treatment group according to 10-year AS CARDIOVASCULAR DISEASE (CVD) risk for patients at the time of enrollment

10-year As Cardiovascular Disease (CVD) risk for patients at the time of enrollment	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Low risk (<5%)	13	8.2	2	3.8
Borderline risk (5%-7.4%)	34	21.4	4	7.5
Intermediate risk (7.5%-19.9%)	67	42.1	8	15.1
High risk (more and equal 20%)	45	28.3	39	73.6
Total	159	100.0	53	100.0

Table 9 (B). Distribution of the treatment group according to their10 year ASCARDIOVASCULAR DISEASE (CVD) risk assessment after three months of enrollment.

10-year Ascardiovascular Disease (CVD) risk for patients after three months of enrollment	Treatment group			
	Test group		Positive control group	
	N	(%)	N	(%)
Low risk(<5%)	65	40.9	7	13.2
Borderline risk (5%-7.4%)	14	8.8	9	17.0
Intermediate risk (7.5%-19.9%)	65	40.9	23	43.4
High risk (more and equal 20%)	15	9.4	14	26.4
Total	159	100.0	53	100.0

Table 10. Distribution of treatment group according to lipid profile (Male)

Lab parameters	Treatment group		P–value
	Test group =69	Positive control group = 30	
Total cholesterol level(mg/dL)			
Baseline Data	241.72±38.11	241.92±31.54	
Data after 1.5-month	227.20±36.47	202.80±25.87	
Data after 3 months	218.24±34.06	174.90±22.87	0.000*
HDL (mg/dL)			
Baseline Data	33.05±3.21	32.00±2.25	
Data after 1.5-month	34.08±3.39	33.13±2.43	
Data after 3 months	34.69±3.13	34.03±2.19	0.000*
LDL (mg/dL)			
Baseline Data	198.27±30.57	196.20±30.91	
Data after 1.5-month	181.07±31.14	143.37±27.16	
Data after 3 months	173.54±29.34	130.30±24.29	0.000*
Triglyceride (mg/dL)			
Baseline Data	280.78±85.81	279.48±115.35	
Data after 1.5-month	230.65±65.60	234.20±66.64	
Data after 3 months	207.07±51.40	141.27±59.55	0.000*

* P–value < 0.05 = Significant

Table 11. Distribution of treatment group according to lipid profile (Female)

Lab parameters	Treatment group		P–value
	Test group =90	Positive control group =23	
Total cholesterol level(mg/dL)			
Baseline Data	244.64±52.18	247.74±37.95	
Data after 1.5-month	228.24±48.10	201.13±31.30	
Data after 3 months	220.12±45.07	175.26±29.54	0.000*
HDL (mg/dL)			
Baseline Data	33.77±3.36	32.22±2.32	
Data after 1.5-month	34.45±3.44	33.26±2.47	
Data after 3 months	35.03±3.23	33.46±2.94	0.000*
LDL (mg/dL)			
Baseline Data	200.32±30.57	197.65±27.89	
Data after 1.5-month	181.07±31.14	143.70±25.47	
Data after 3 months	173.54±29.34	130.91±22.04	0.000*
Triglyceride(mg/dL)			
Baseline Data	272.32±99.69	271.57±94.52	
Data after 1.5-month	210.92±74.24	161.04±53.43	
Data after 3 months	195.25±60.68	142.00±50.88	0.000*

* P–value < 0.05 = Significant

Table 2 indicates the Mean age and SD distribution of the treatment group according to gender in both the treatment and control groups. Table 3 shows the Distribution of the treatment group according to gender where 56.6% are men and 43.4% female. Table 4 shows the distribution of the treatment group according to Occupation, where Service holders 39.6% and Housewives 32.1% most of the numbers. Table 5 shows the distribution of the treatment group according to Diabetes where Type I DM and Type II DM are more prevalent respectively. Table 6 shows the distribution of treatment groups according to treated hypertension and found that most of the patients in both groups are not treated with hypertension 78.6% and 79.2% respectively in the test and control groups. Table 7 shows the distribution of treatment groups according to smoking history we found most of the participants are non-smokers 73.6% and 58.5% of both test and control groups. Table 8 ındicating the distribution of the treatment group according to Body Mass Index and we found that most of the population 61.0% in the Test group were overweight. Table 9(A) shows the distribution of the treatment group according to 10-year As Cardiovascular Disease (CVD) risk for patients at the time of enrollment, Table 9 (B) presents the Distribution of the treatment group according to their year As Cardiovascular Disease (CVD) risk assessment after three months of enrollment and we found most of the population is under Intermediate risk (7.5%-19.9%).

Response on HDL Cholesterol levels of test group

159 individuals received the test medication, 69 of them were men and 90 of whom were women. The baseline mean HDL level for male responders was 33.05±3.21 mg/dL. As demonstrated in Table 10, the HDL level increased after six weeks of medication treatment to 34.08±3.39 mg/dL and after twelve weeks to 34.69±3.13 mg/dL. The baseline mean HDL level for female responders was 33.77±3.36 mg/dL. As indicated in table 11, the HDL level rose to 34.45±3.44 mg/dL and 35.03±3.23 mg/dL after 6 weeks and 12 weeks of medication delivery.

Response of Test and Control drug in LDL Cholesterol levels

159 individuals received the test medication, 69 of them were men and 90 of whom were women. The baseline mean LDL level for male responders was 198.27±30.57 mg/dL. As demonstrated in table 10, the LDL level decreased after six weeks of medication treatment to 181.07±31.14 mg/dL and after twelve weeks to 173.54±29.34 mg/dL. The baseline mean LDL level for female responders was 200.32±30.57 mg/dL. As indicated in table 11, the LDL level decreased to 181.07±31.14 and 173.54±29.34 mg/dL after 6 weeks and 12 weeks of medication delivery.

Response of Test and Control drug in triglycerides levels

At baseline, the mean triglyceride level in 69 male patients receiving the test medication was 280.78±85.81 mg/dL, while the mean level in 30 male cases receiving the control medication was 279.48±115.35 mg/dL. As indicated in table 10, total triglyceride levels were decreased after 12-weeks of medication treatment to 207.07±51.40 mg/dL from the baseline in the test group and 141.27±59.55 mg/dL in the positive control group. At baseline, the mean triglycerides level in the test group of 90 female patients was 272.32±99.69 mg/dL, while the mean level in the positive control group of 23 female cases was 271.57±94.52 mg/dL. According to table 11, total triglyceride levels in the test group were lowered from baseline values to 195.25±60.68 mg/dL and to 142.00±50.88 mg/dL in the positive control group following the 12-week medication treatment.

Discussion

One research examines how garlic affects people with type 2 diabetes mellitus who have dyslipidemia, one of the main cardiovascular risk factors. The findings demonstrate that garlic significantly decreased total cholesterol (-28 mg/dl, -12.03% P= 0.001) and LDL-C (Low-Density Lipoprotein) (-30 mg/dl, - 17.99% P= 0.001), whereas the placebo-treated group (n=32) experienced a non-significant reduction in total cholesterol (-2 mg/dl, - 0.9% P= ns) and LDL-C (Low-Density Lipoprotein) (-3 mg/dl, - 1.6% P= n.s). Patients receiving garlic therapy had substantially higher HDL cholesterol (3.35 mg/dl, 8.81% P= 0.05) compared to the placebo group (0.62, 1.6% P= n.s.), but there was no discernible change in triglyceride levels between the two groups. The results show that, as compared to a placebo, garlic dramatically decreased blood total cholesterol and LDL cholesterol and somewhat increased HDL cholesterol (Ashraf et al., 2005). In another study, the impact of garlic powder at 5% and 10% concentrations on the plasma lipid profile in hypercholesterolemic rats was examined. In order to induce hypercholesterolemia, male albino rats were given a diet that included 20% fat and 1% cholesterol for two weeks. The treated groups' plasma total cholesterol and LDL-C (Low-Density Lipoprotein) levels were considerably lower than those of the hypercholesterolemic control group, according to the results- HDL-C (High-Density Lipoprotein), however, showed a substantial improvement ($P<0.05$) (Ajayi et al., 2014). This double-blind randomized, placebo-controlled intervention study was conducted in 46 hypercholesterolemic subjects who had failed or were noncompliant with drug therapy to assess the hypocholesterolemic effect of an enteric-coated garlic supplement standard for allicin-releasing potential in mild to moderate hypercholesterolemic patients. Each participant received dietary advice to reduce fat consumption, enteric-coated Australian garlic powder pills with a possible allicin release of 9.6 mg, or identical placebo tablets. After 12 weeks, it was shown that those who took garlic supplements significantly reduced their total cholesterol and LDL cholesterol, whereas those who took placebos saw no significant change in their TC, LDL-C (Low-Density Lipoprotein), or HDL levels (Kannar et al., 2001). The cardiovascular system's response to pure allicin was examined in the study. To achieve this, 20 naturally hypertensive rats were given a daily dosage of pure allicin added to their food for six weeks, as compared to control rats that received standard food. At baseline and at the conclusion of the trial, measurements of weight, systolic blood pressure (SBP), triglycerides, cholesterol, insulin, and adiponectin were made. Allicin had no impact

on body weight, however it dramatically lowered triglyceride levels and SBP, dropping SBP from 190 mmHg to 168 mmHg (P<0.0001) and 96 mmHg to 71 mmHg (P=0.009), respectively. Plasma levels of adiponectin, insulin, and cholesterol were unaffected by allicin (Elkayam et al., 2013). The purpose of the study was to determine how Allium sativum affected guinea pigs with experimentally induced hyperlipidemia. 25 guinea pigs were fed cholesterol (0.5 g/Kg body weight/day) for this purpose during the course of an initial 4-week period. The findings demonstrated that garlic's aqueous and alcoholic extracts significantly lowered blood triglycerides, LDL cholesterol, VLDL cholesterol, and atherogenic index in hyperlipidemic guinea pigs (P<0.001) compared to the control group. Animals in group II showed a substantial increase in HDL-C (High-Density Lipoprotein), whereas those in groups I and III did not. A comparison of the two extracts revealed that the aqueous garlic extract was a more effective hypolipidemic medication than the alcoholic extract (Choudhary et al., 2013).

Diallyldisulphide (DADS), an unsaturated aliphatic disulphide, is the major sulphur component found in garlic extract and garlic oil and is regarded to be primarily responsible for the health benefits of garlic. In the current study, rats given a high-lipid diet (HLD) were used to test the efficacy and toxicity of garlic extracts. The findings show that garlic aqueous extracts in high cholesterol diet rats cause fatty liver alterations and hypolipidemic effects in plasma. The main sulfur component in garlic, DADS, may be the cause of these hypolipidemic effects. (Murthy, 2014). Researchers found that supplementing with aged garlic extract (AGE) reduced plasma concentrations of total cholesterol and LDL cholesterol in hypercholesterolemic men by 7% and 10%, respectively, compared to subjects receiving a placebo. This was demonstrated in a randomized, double-blind, placebo-controlled intervention study (Yeh and Liu, 2001). Garlic's capacity to lower blood total cholesterol, LDL and LDL oxidation, platelet aggregation, and hypertension has been demonstrated in several in vitro experiments. It has been demonstrated that garlic inhibits lipid synthesis-related enzymes, reduces platelet aggregation, stops lipid peroxidation of damaged erythrocytes and LDL, boosts antioxidant status, and blocks angiotensin-converting enzymes (Rahman and Lowe, 2006). Consuming garlic promotes fat metabolism and lowers blood cholesterol levels. protects blood arteries and the heart by raising levels of "good" cholesterol HDL and lowering levels of "bad" LDL cholesterol and triglycerides (Majewski, 2014). On the other hand, a little Indian research with 32 hypercholestermic participants combined fish oil use with garlic consumption. With the exception of high-density lipoprotein, which rose, all lipid indicators showed a substantial decrease for the test group. After taking supplements for 60 days, blood triglycerides, very low-density lipoprotein, total cholesterol, and low-density lipoprotein all decreased by 20%, 21%, 37%, 36.7%, and 23.4%, respectively. High-density lipoproteins that are protective went increased by 5.1%. When taken with other antioxidants like lycopene and vitamin E, the potential of garlic may be increased (Bongiorno et al., 2008). When compared to the control group, those who consumed garlic had significantly lower triglyceride (TG) and total cholesterol (TC), the two primary risk factors for arteriosclerosis. When compared to the control group, the administration of 1000 mg of garlic reduces low density lipoprotein cholesterol (LDL-C (Low-Density Lipoprotein)) levels while increasing high density lipoprotein cholesterol (HDL-C (High-Density Lipoprotein)) levels and very low density lipoprotein cholesterol (VLDL-C (Low-Density Lipoprotein)). Therefore, it may be said that garlic may be quite helpful in treating patients who have dyslipidemia (Hussien, 2014). (Brüll et al.,2015) Effects of an onion skin extract high in quercetin on endothelial function and 24-hour ambulatory blood pressure in individuals with (pre-) hypertension who are overweight to obese. Additionally, the World Health Organization endorses the use of onions to cure and prevent atherosclerosis and appetite loss. Similar to garlic, regular onion consumption decreases blood pressure, serum triglyceride and cholesterol levels, while raising HDL levels. As a result, it lowers the risk of heart attacks and strokes and avoids atherosclerosis and diabetic heart disease (Kumar et al., 2010). (Lee et al., 2008) In their investigation on the impact of dietary supplementation with onion powder on lipid metabolism in high fat-cholesterol fed SD In hyperlipidemic rats, it was shown that feeding them onion powder prevented weight growth, markedly reduced the amount of total cholesterol in the liver, and restored GOT function. In rats with Triton X-100-induced hyperlipidemia, ethanolic extracts of Syzygium cumini at doses of 200 and 400 mg/kg significantly reduced hyperlipidemia. Because this component may lower total cholesterol and total triglyceride levels in rats, it was discovered that flavonoids, triterpenoids, and tannins are the active plant elements that were responsible for the anti-hyperlipidemic effect (Singh et al., 2018). After using S. cumini seed powder for 60 days, dyslipidemia has improved (Sidana et al., 2016). In chronic restraint stress mice, the injection of an ethanol extract of S. cumini (L.) pulp considerably reduced the rise in blood pressure (P<0.001). Additionally, the treatment groups' MDA levels were considerably lower than those of the negative controls (P<0.05), showing that an ethanol extract from S. cumini (L.) pulp may stop the rise in MDA levels (Suryajayanti et al., 2017). Diet high in cholesterol Rats showed a statistically significant rise in serum triglycerides, low density lipoproteins, very low density lipoproteins, and the atherogenic index, as well as a statistically significant drop in the ratio of high density lipoproteins to low density lipoproteins (Modi Dikshit et al., 2009). A standardized extract for hypocholesterolemic activity was developed from the leaves of Mangifera indica as a result of a study on the Cholesterol esterase inhibitory activity of bioactives from Mangifera indica L. leaves. The study discovered that the methanolic extract of Mangifera indica leaf had significant anticholesteremic activity (Gururaja et al., 2015). Another study on the impact of ethanol leaf extract of Mangifera indica on the lipid profile of alloxan-induced diabetic albino rats revealed the plant's leaf extract has anti-hyperlipidemic properties, pointing to its potential for treating hyperlipidemia and its associated cardiovascular complications (Ezeani et al., 2017).

Mangifera indica Leaves extract had considerable antihyperlipidemic activity as well as renoprotective and hepatoprotective effects in diabetic albino rats, according

to the study on Assessment of The Therapeutic Role of Mangifera indica Leaves Extract in Diabetic Albino Rats. (Maghfur et al., 2022).

Natural treatments for hyperlipidemia: According to a review, the extract from Mangifera indica leaves acts to increase the expression of hepatic LDL receptors, protect against LDL-C (Low-Density Lipoprotein) buildup in the blood, and catabolize cholesterol from the body's LDL receptors (Dasgupta et al., 2021).

(Arulmozhi et al., 2007) discovered that Myristica fragrans extract effectively decreased the raised TG (47% reduction at 450 mg, $P<0.01$) and cholesterol (66.7% reduction at 450 mg, $P<0.01$) in rats fed a high-cholesterol diet. The extract also demonstrated a reduction in hepatic TG production following tyloxapol treatment. (Vangoori, Y et al., 2019) observed that the concentrations of TC, TD, LDL, and VLDL were substantially ($P<0.05$) and dosage dependently lowered by the ethanolic extract of Myristica fragrans, whereas the concentrations of HDL were raised.

(Kareem et al., 2009) in their study indicated small reductions in the levels of cholesterol (11.0%), triglycerides (21.7%), FFA (53.7%), and PL (10.6%) were seen in rats pretreated with nutmeg extract and then administered isoproterenol. These values were kept at or close to normal by nutmeg extract pretreatment and were 1.86%, 5.54%, 5.65%, and 0.30% for cholesterol, triglycerides, FA, and PL, respectively. (Pashapoor et al., 2020) discovered that giving nutmeg extract to diabetic rats (100 and 200 mg/kg) significantly decreased their levels of malondialdehyde, total cholesterol, triglycerides, and low-density lipoprotein while significantly increasing their levels of high-density lipoprotein cholesterol and total antioxidant capacity. (Sompong et al., 2016), demonstrated that *Syzygium aromaticum* significantly suppressed pancreatic cholesterol esterase activity 1.07% at a dosage of 1 mg/mL. Additionally, they discovered that Syzygium aromaticum had values ranging from 2.29 to 33.74% that marginally inhibited the development of cholesterol micellization. (Nethrakere et al 2015) discovered that as compared to the dexamethasone control group, the clove oil treated groups had significantly lower total cholesterol and triglyceride levels and higher HDL levels ($P<0.01$). The values of HDL, total cholesterol, and triglycerides in the pioglitazone group and the clove oil group, respectively, were comparable ($P = 0.167$, $P = 0.159$, and $P = 0.278$).

Maraia (2014), discovered that clove oil-both fixed and volatile-reduced blood lipid parameters and MDA level, and that this impact was linked to an increase in antioxidant enzyme levels and HDL level.

Previous studies on the benefits of garlic alone have mostly focused on lipid levels. One key difference between our study and several other investigations was the use of garlic in combination with other healthful plants. Only 20 g of garlic and 1 tablespoon of lemon juice were prescribed to the patients, however the aforementioned elements had some noticeable effects. Dry extracts of onion (Allium cepa), black plum (Syzygium cumini), mango (Mangifera indica), nutmeg (Myristica fragrans) fruit, and clove (Syzygium aromaticum) flower were given to research participants, but no particular diet was suggested. When assessing the study's findings, this constraint must be considered. Dietary history for the patient was reviewed.

Conclusions

In conclusion, those with hyperlipidemia have lower lipid levels after taking a combination of dry extracts from the following plants: garlic (Allium sativum), onion (Allium cepa), black plum (Syzygium cumini), mango (Mangifera indica), nutmeg (Myristica fragrans), and clove (Syzygium aromaticum). To determine the appropriate diet and nutritional state of patients, more study is required. Additionally, depending on the degree of the hyperlipidemia, the effects of a combination of dry extracts from the following plants were studied: clove (Syzygium aromaticum) flower, black plum (Syzygium cumini) seed, mango (Mangifera indica) leaf, nutmeg (Myristica fragrans) fruit, and garlic (Allium sativum) dry extract. Accordingly, research is needed to ascertain the effects of dry extracts of the following plants on hyperlipidemia: dried clove flower (Syzygium aromaticum), dried black plum seed (Syzygium cumini), dried mango leaf (Mangifera indica), dried nutmeg fruit (Myristica fragrans), and dried onion (Allium sativum).

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Comprehensive Evaluation of the Clinical Efficacy of an Anti-Diabetic Polyherbal Formulation

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ABSTRACT

Background: There are several clinical studies examining the health advantages of several single medicinal herbs utilized in traditional blood glucose-lowering treatments. But very few or no studies on herbal formulations were made as Polyherbal for the same goal. As a result, it is now necessary to confirm that patients with hyperglycemia can benefit from such Polyherbal medicines as Dolabi. *Methods:* This prospective open-label, herbal coded test drug-controlled, randomized trial was conducted at the Munshiganj and Dhaka area in Bangladesh. We enrolled 108 male and 104 female patients of 30-70 years with primary and moderate hyperglycemia. They were recruited from the OPD of an Unani & Ayurvedic hospital in Munshiganj and different Unani clinics in Dhaka, Bangladesh after fulfilling the inclusion criteria. Patients were randomly assigned to receive metformin hydrochloride 500 mg two times daily and 2 tablets of Dolabi two times daily by using a random numbers table with the help of an assistant. Blood samples, height, weight, blood pressure, and personal data were recorded—laboratory results were obtained at the study baseline, after 1.5 months and after 3 months of intervention. *Results:* In the case of the test drug, results showed a significant decrease in blood glucose level between the baseline and after 3 months, in males, it was from 9.83±1.17 to 7.72±1.06 mg/dL for fasting glucose, from 16.60±2.35 to 8.23±1.17 mg/dL for 2 hours PP glucose, from 9.33±1.17 to 7.45±2.03 percent for HbA1c and for Insulin it reduces from 183.10±27.59 to 168.10±29.59 pmol/ L. The control drug metformin hydrochloride also showed a significant decrease in blood glucose level between baseline and after 3 months, in the case of males it was from 9.99±2.52 to 6.97±1.76 mg/dL for fasting glucose, from 17.43±5.05 to 7.89±2.42 mg/dL for 2 hours PP glucose, from 10.43±2.36 to 6.87±1.18 percent for HbA1c and for Insulin it reduces from 198.75±30.61 to 183.75±30.61p mol/L. In the case of females the test drug showed a significant reduction in fasting glucose, 2 hours PP glucose, HbA1c and Insulin between the baseline and after 3 months, it was from 10.02±1.11 to 7.78±0.93 mg/dL, from 16.88±2.21 to 8.16±1.11 mg/dL, from 9.84±1.04 to 7.45±1.03 percent and from 199.47±30.90 to 173.47±30.90 mg/dL respectively. In the case of females, the control drug showed a significant reduction in fasting glucose, 2 hours PP glucose, HbA1c and Insulin between baseline and after 3 months, it was from 10.18±1.92 to 6.71±1.59 mg/dL, from 18.70±3.88 to 7.60±3.74 mg/dL, from 10.58±1.08 to 6.98±1.08 percent and from 200.00±31.83 to 188.00±31.83 mg/dL respectively. *Conclusions:* We can infer the following from the present study's findings: The polyherbal formulation Dolabi is able to reduce the blood glucose level. It can be an effective drug for primary hyperglycemic patients.

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Introduction

Patients who have diabetes are at a greater risk of developing chronic metabolic disorders, including high blood glucose (also known as blood sugar) levels, which over time can have a detrimental effect on their hearts, blood vessels, eyes, kidneys, and nerves. According to the Global Burden of Disease Collaborative Network 2020, type 2 diabetes is the most prevalent form of the disease and develops when the body either stops producing enough insulin or becomes insulin resistant (Global Burden of Disease Collaborative Network 2020). This form of diabetes most commonly affects adults. Over the past three decades, there has been a marked increase in the prevalence of type 2 diabetes in nations with incomes ranging from very low to very high. When the pancreas produces very little or none of its own insulin, a person is said to have the chronic illness known as diabetes type 1. Juvenile diabetes or insulin-dependent diabetes where its earlier titles (Diabetes, 2023). It is essential for diabetes patients to have

access to reasonably priced medical treatment, particularly insulin, in order for them to have any chance of survival. According to 'Diabetes- Overview' (n.d.), all parties have reached a consensus that an end must be put to the rise of diabetes and obesity by the year 2025 ('Diabetes- Overview' n.d.). The bulk of the world's 422 million diabetics, who together live in low- and middle-income nations, are afflicted with the disease, which is directly responsible for 1.5 million deaths each year. Over the course of the last few decades, both the incidence of diabetes and the prevalence of diabetes have seen a steady increase ('Diabetes- Overview' n.d.).

Diabetes affected 8.5% of those aged 18 and older in 2014 in the United States. In 2019, diabetes was directly responsible for the deaths of 1.5 million individuals over the world, with those under the age of 70 accounting for 48 percent of these fatalities. According to the Global Burden of Disease Collaborative Network 2020, hyperglycemia is responsible for twenty percent of all fatalities from cardiovascular disease, and diabetes is responsible for an additional four hundred and sixty thousand deaths from renal sickness. The age-standardized death rates for diabetes have increased by 3% between the years 2000 and 2019. According to Diabetes, 2023, nations with lower-middle incomes had a 13% rise in the death rates caused by diabetes. On the other side, the risk of dying between the ages of 30 and 70 from any of the four most common noncommunicable illnesses (cancer, chronic respiratory diseases, diabetes, or cardiovascular diseases) reduced by 22% throughout the globe between the years 2000 and 2019 (Diabetes, 2023).

According to the International Diabetes Federation (IDF), Bangladesh ranked seventh globally in 2021 with 13.1 million cases of adult diabetes (ages 20-79) (International Diabetes Federation, 2019) Our forecasts indicate that Bangladesh will hold the seventh position in the world by the year 2045.

One of the seven countries that make up the IDF SEA area, Bangladesh is one of them. According to 'The International Diabetes Federation (IDF) Bangladesh, the number of people living with diabetes is expected to rise to 151.5 million by the year 2045(The International Diabetes Federation (IDF)Bangladesh, n.d.). There are now 537 million people living with diabetes in the world. According to the findings of the study, the number of diabetics in Bangladesh would rise to over 7.9 million by the year 2020, which is about one in ten adults in the country. It should be underlined that our data included a younger demographic than the estimates provided by the IDF; as a result, the total case count is inflated due to the fact that diabetes is less prevalent in the younger age range. Despite this, there is an immediate need for policies that will support the implementation of diabetes prevention programs in this country. This is due to the high incidence of diabetes cases in Bangladesh, which places it among the nations of Southeast Asia with the largest burden of the illness (Hossain et al., 2022). Both diabetes and its precursor, prediabetes, affect a sizeable percentage of the population in Bangladesh. According to the numbers from the most recent BDHS 2017–18 survey, in the year 2020, more than 19 million Bangladeshis who were 18 years of age or older will have diabetes or prediabetes. Diabetes was connected with age, sex, body mass index (BMI), income quintile, job status, hypertension, and the administrative division of the nation; however, it was not associated with either location of residence (urban or rural) nor degree of education. These findings provide further evidence that diabetes and prediabetes continue to have a pervasively high incidence in Bangladesh (Hossain et al., 2022).

Throughout the course of human history, several communities have placed a significant amount of their medicinal reliance on plants and herbs (''Medicinal Plants and Herbs for Diabetes. n.d.). The treatment and management of diabetes is now the focus of research in contemporary medicine, which is looking at the viability of using traditional remedies either on its own or in conjunction with conventional pharmaceuticals. It is imperative that the origin and purity of a plant be established before determining whether or not it is effective and whether or not it may mitigate any potential adverse effects. Nazamuddin et al. (2014), state that if employing herbal medications, one should always seek the opinion of a qualified specialist (Nazamuddin et al., 2014).

In the polyherbal Unani preparation Tablet DOLABI®, Have a combination of three great antidiabetic herbs as well as one mineral. In addition to Gymnema *(Gymnema sylvestre)*, Bamboo Manna *(Bambusa bambos)*, Bladder Dock *(Rumex vesicarius)*, and Mineral Pitch (Shilajit), the anti-diabetic mixture also contains Bladder Dock *(Rumex vesicarius)*. Dolabi is a research product that is manufactured by Hamdard Laboratories. It is a tried-and-true cure for diabetics that comes from the Unani tradition. The most important ingredient in Dolabi is gymnema, also known as Gymnema sylvestre. Additionally, it is referred to as gurmar, which is a Hindi word that literally translates as "destroyer of sugar" (Tiwari et al., 2017). Insulin secretion is improved with dolabi, and the drug also improves pancreatic function. It does this by aiding in inhibiting the absorption of sugar from the gastrointestinal tract (''Tablet Dolabi-Description'' n.d.). This helps to keep a normal blood sugar level, which is beneficial to overall health.

Gymnema (Gymnema sylvestre)

There is a kind of woody climbing plant known as Gymnema sylvestre that is native to the tropical woods in the middle and southern parts of India. In the production of herbal treatments, the leaves constitute an essential component. Meshasringi is the Sanskrit name for G. sylvestre, which translates to "ram's horn" in English and "periploca of the woods" in Spanish. Both of these names relate to the same plant. The chewing of the leaves affects one's capacity to sense sweetness, which is where the Hindi word gurmar, which translates to "destroyer of sugar," comes from (Tiwari et al., 2017).

It wasn't until the late 1920s that researchers discovered the hypoglycemic (or blood sugar-lowering) properties of gymnema leaves (Mhaskar and Caius, 1930). It is assumed that compounds belonging to the chemical family known as gymnemic acids are the ones responsible for the pharmacological action. According to research conducted by Sugihara et al. (2000) on healthy participants, gymnema leaves cause a rise in insulin levels (Sugihara et al., 2000). Research conducted on animals suggests that this might be occurring for one of two reasons: either the insulin-secreting cells in the pancreas are renewing (Shanmugasundaram et al., 1090; Prakash et al., 1986) or the insulin flow from these cells is growing.

Figure-1. *Gymnema sylvestre* fresh leaf. source. Wikipedia

Figure-2. *Gymnema sylvestre* dry leaf.

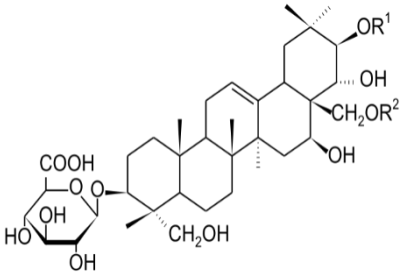

Figure-3. Gymnemic Acids source. Wikipedia

Figure-4. *Bamboo Manna (Bambusa bambos)* Tree. Source. Wikipedia

Figure-5. Bamboo shoot Source. Wikipedia

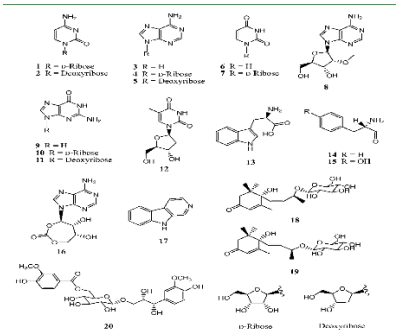

Figure-6. Chemical Constituents of *Bamboo Manna* source. (Sun, J et al.,2016).

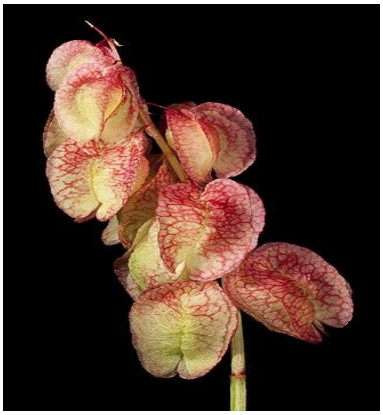

Figure-7. Bladder Dock (Rumex vesicarius) flower Source. Wikipedia

Figure-8. Bladder Dock herb Source. Wikipedia

Figure-9. Structures of Luteolin source. (Al-Masri, A. A et al.,2023)

Figure-10. Mineral Pitch (Shilajit) In mountain source. https.//mapi.com

Figure-11. Mineral Pitch (Shilajit) Rock source.https.//www.tattvasherbs.com

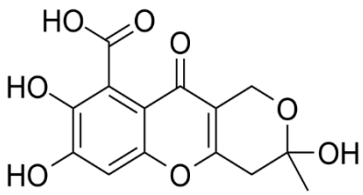

Figure-12. Fulvic Acid source. Med Chem Express

According to other animal research (Gholap and Kar, 2003, Shimizu et al., 1997), gymnema has the ability to bring blood sugar levels down by increasing the amount of glucose that is taken up into cells, preventing adrenal hormones from stimulating the liver to produce glucose, and reducing the quantity of glucose that is absorbed from the stomach. In the clinical studies, the use of gymnema sylvestre as an anti-diabetic medication was proven to be successful. The action that is insulinotropic Adult human volunteers (25–40 years old) displayed activity after taking two doses of 2 g/day of Gymnema sylvestre (Shanmugasundaram et al., 1981). When given to ten healthy subjects over the course of ten days and to six diabetics over the course of fifteen days, a water-based Gymnema sylvestre leaf extract may have decreased fasting and oral glucose tolerance test (OGTT) glucose

intensity (Khare et al., 1983). The only exception to this was the OGTT in the normal cluster. It has been established that a diabetic patient's HbA1C level may be lowered by ingesting 400 mg of the leaf extract of Gymnema sylvestre twice a day (Joffe and Freed, 2001). Figure 1, 2 and 3 is showing the picture of: fresh leaf of Gymnema sylvestre, Gymnema sylvestre dry leaf, Gymnemic Acids.

***Bamboo Manna* (*Bambusa bambos*)**

Bambusa bambos is a species of clumping bamboo that is endemic to Southern Asia. It is also known as the huge thorny bamboo, Indian thorny bamboo, spiny bamboo, and thorny bamboo. It may be found in India, Bangladesh, Sri Lanka, and Indochina. In addition to this, it has been granted citizenship in the countries of the Seychelles, the Philippines, Java, Malaysia, Maluku, Central America, and the West Indies. (Ohrnberger, 1999). Figure 4, 5 and 6 is the picture of: *Bamboo Manna (Bambusa bambos)* Tree, Bamboo shoot and, Chemical Constituents of Bamboo Manna.

In yet another study, streptozotocin-induced diabetic rats were given bambusa arundinacea, which was reported to have hypoglycaemic effects (Nazreen et al., 2011). They found in their research that treating rats resulted in a significant decrease in blood sugar levels, as well as a fall in glutathione and lipid peroxidation levels, and an increase in enzyme activity. These changes were all brought about by the treatment. According to their results, diabetic mice are susceptible to oxidative stress, and the leaf extract of B. balcooa has the potential to aid in reestablishing a healthy balance between the processes that lead to the production of reactive oxygen species and the activity of enzymes that assist scavenge them. (Goyal et al., 2017).

***Bladder Dock* (*Rumex vesicarius*)**

Rumex vesicarius is a species of perennial flowering plant that belongs to the family Polygonaceae. It is also known by the common name's bladder dock and Ruby dock, among other names. Rumex vesicarius is an indigenous plant that may be found in tropical and temperate regions of Asia, Africa, and Western Australia, according to Plants of the World Online. (Rumex vesicarius L, 2020). Figure-7, 8 and 9 is the picture of: *Bladder Dock (Rumex vesicarius)* flower, Bladder Dock herb and Structures of Luteolin.

The leaves of Rumex vescarius L. are used to provide local treatment for diabetes, a disease that is chronic. The flavonoid luteolin from Rumex vescarius was chosen to study for the possibility of acting as an anti-diabetic agent through the use of an in vivo test against male albino Wistar rats. These rats had been fed alloxan to induce diabetes. Interaction with the enzyme alpha-glucosidase may be responsible for the potent anti-diabetic properties of the plant and the flavonoid luteolin that the plant has (Al-Masri et al., 2023).

***Mineral Pitch* (Shilajit)**

It is an exudate that is frequently found in the Himalayas, Badakhshan in Afghanistan, the Karakoram, Gilgit-Baltistan in Pakistan, Nepal, Bhutan, Russia, Central Asia, Iran, Mongolia, and the south of Peru, where it is known as Andean Shilajit. Shilajit is also known as Mumijo (Wilson et al., 2011). Shilajit is also known as Mumijo. Mumijo is another name for Shilajit. (Hill and Forti, 1997). Figure-10,11 and 12 is showing the picture of: Mineral Pitch (Shilajit) In mountain, Mineral Pitch (Shilajit) Rock and chemical structure of Fulvic Acid.

The effects of Shilajit extract on diabetic neuropathy in streptozotocin-induced diabetic male rats were explored utilizing behavioral and cytotoxic activities. The doses used were fifty, one hundred, and two hundred milligrams per kilogram. After administering Shilajit injections at a dose of 100 milligrams per kilogram per day for a period of four weeks, researchers (Trivedi et al., 2004) discovered that blood sugar levels fell as a result of the treatment. Shilajit, at a dose of 100 mg/kg, was orally administered to the animals, and it was determined that this resulted in lower levels of blood sugar (Bhattacharya, 1995). This finding was made in comparison to the group that served as the control. According to the findings of a study carried out by Trivedi et al. (2004), reactive oxygen species have a substantial role in the progression of diabetic neuropathy. Shilajit has the capacity to scavenge free radicals, which decreases the harmful impact that accumulated free radicals can have on pancreatic cells (Bhattacharya, 1995). According to the findings of an experiment (Bhattacharya, 1995), Shilajit has the ability to scavenge free radicals.

Methods

Type of the study: In this prospective, open-label, herbal coded test drug-controlled, parallel trial, allopathic (Metformin HCL 500mg) and herbal coded test drug groups (Tablet DOLABI®) were allocated in a 3:1 ratio.

Study population: Participants in the research must be between the ages of 30 and 70, be of both sexes, have fasting blood glucose (FBG) > 5.6 mmol/L or HbA1c > 7%, and have been using oral hypoglycaemic medications (metformin and/or glibenclamide) for more than three months.

Sample size: Patients were picked and registered by a process known as random sampling. Screening tests, such as blood sugar levels while the patient was fasting and lipid profiles, were performed on an average of two to three patients every clinic day. The clinical experiment had a total of 212 participants' participation. Following the completion of the inclusion and exclusion procedures, the test and positive control groups comprised a total of 159 patients and 53 patients, respectively.

Sampling technique: Respondents were randomly assigned to receive either the herbal product or the intervention. Participants were randomly assigned to receive either the Dolabi or the Metformin HCL formulation using computer-generated randomization. Each supplement has a code correlating to the trade name box. Each and every supplement was enclosed in a set of uniform, transparent capsules created by the respective pharmaceutical firm. Participant recruitment, gathering information, and analysis (MM), as well as the supplements each participant gets, were all known to the participants and investigator.

Inclusion criteria: Participants in this research have to meet the requirements listed below: (1) Were diagnosed as T2D at least for 2 years duration; (2) Were > 30 years old; (3) Had low density lipoprotein (LDL100 mg/dL; (4) Triglycerides 150 mg/dL; (5) Had been receiving hypolipidemic (statins) drugs for over three months; (6) Had fasting blood glucose (FBG) > 5.6 mmol/L or HbA1c > 7% (53 mmol/mol); (7) Had been receiving oral hypoglycaemic drugs (metformin and/or glibenclamide) for over three months.

Exclusion criteria: Participants were deemed ineligible if they fulfilled any one of the following requirements: (1) They had type 1 diabetes, gestational diabetes, or another specific type of diabetes; (2) They were candidates for insulin therapy; (3) They had used other herbal supplements for diabetes control during the course of the study; (4) They had severe renal or hepatic impairment; (5) They had severe infection; (6) They had a history of allergies to PHF plants; (7) They were addicted to alcohol or drugs; and (8) They were pregnant or nursing.

Data analysis: In addition to descriptive statistics, mean and standard deviation calculations were performed. The identification of connections between socio-demographic parameters was accomplished through the use of cross tabulation and chi square statistics. The data were analyzed with SPSS (version 18, SPSS, Chicago, Illinois, USA), and a two-sided P value of 0.05 was considered to be significant. SPSS was distributed in the United States. In order to measure parameters of metabolic profiles, blood samples were obtained at the beginning of the experiment to establish a baseline, after 1.5 months of intervention, and after 3 months of intervention. A two-tail paired t-test was carried out as part of the main effect research in order to evaluate the usefulness of both the baseline data and the data collected after three months of blood glucose monitoring. In order to investigate the overall characteristics of the baseline, a Chi-square test was carried out on the categorical data. After obtaining a significant result from the two-tail paired t-test, we carried out Tukey's post hoc comparisons in order to establish whether or not there were pairwise differences.

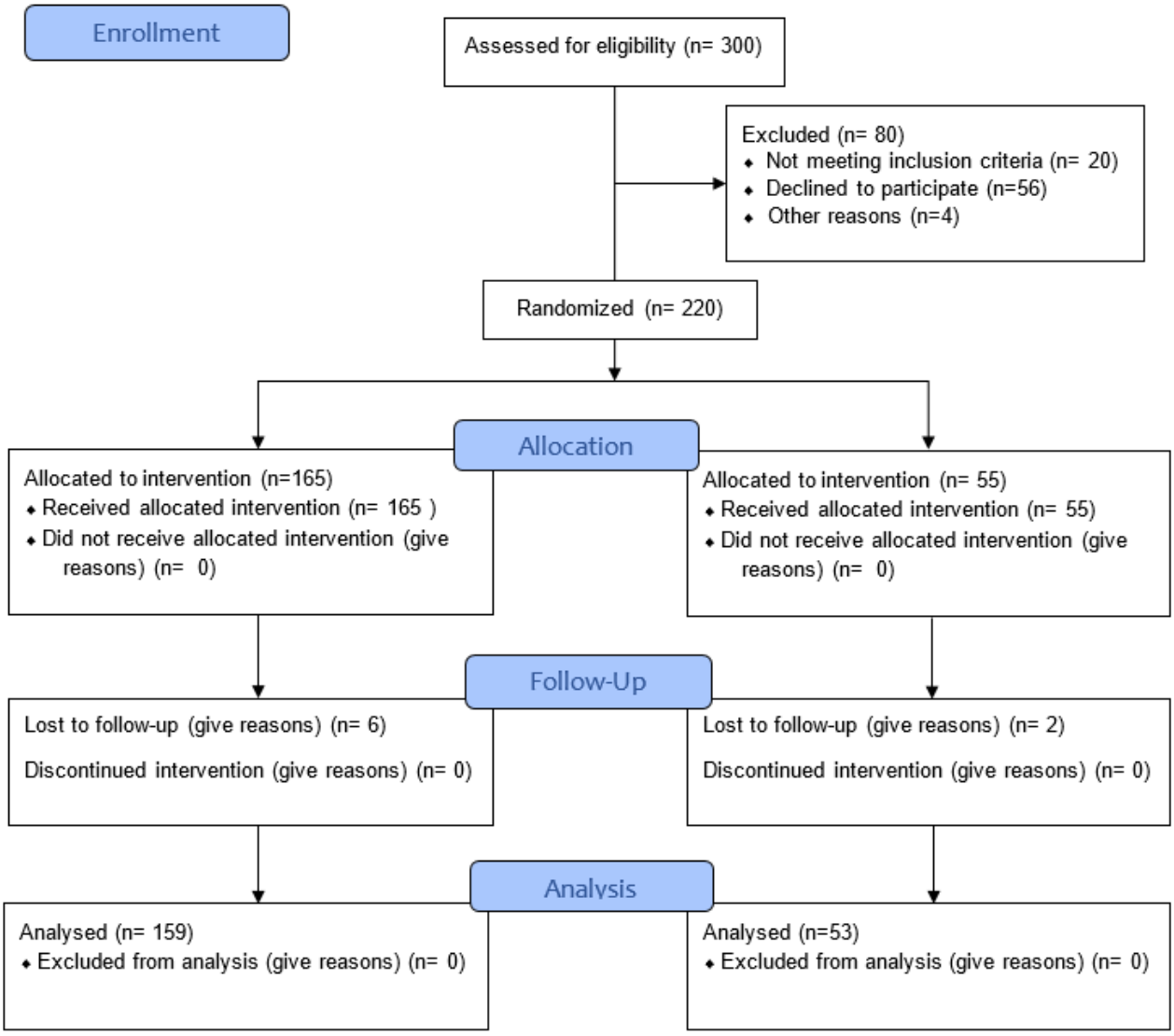

Figure 13: RCT Flowchart

Results

According to the results of our recent study, the majority of male diabetes patients of test group are in 36 and 45 class and for positive control group the class is 46-55 years, in case of female respondents' majority of diabetes patients in both groups are between the ages of 36 and 45 showed in Table 1. Participants are a sizable number of service bearers 45 showed in Table 2. The majority of participants in both groups had comorbid conditions 45 showed in Table 3. The majority of the study group was overweight, with a BMI between 23.0 and 27.5, and they had a history of diabetes in their families 45 showed in Table 4 and 5. From 1 to 5 years, the majority of the population is suffering from diabetes 45 showed in Table 6. Table 7 shows that, among 80 male respondents of test group and 28 male respondents of positive control group, mean of fasting glucose level at baseline were 9.83±1.17 and 9.99±2.52 respectively, after 1.5- month the mean was 8.61±1.07 in test group and 8.98±2.52 in positive control group and after 3 months mean was in test group 7.72±1.06 and in positive control group 6.97±1.76. Regarding 2 hours PP glucose level, among 80 respondents of test group and 28 respondents of positive control group, mean at baseline were 16.60±2.35 and 17.43±5.05, after 1.5-month mean was in test group 10.00±1.17 and in positive control group 8.29±2.42, after 3 months mean was in test group 8.23±1.17 and positive control group 7.89±2.42. Regarding HbA1c level, the mean was 9.33±1.17 and 10.43±2.36 at baseline, 8.43±1.17 and 8.35±2.03 after 1.5-month and 7.45±2.03 and 6.87±1.18 after 3 months in test and positive control group respectively. Regarding insulin level, at baseline the mean were 183.10±27.59 and 198.75±30.61. After 1.5-month they were 175.10±27.59 and 190.75±30.61 and after 3 months they were 168.10±29.59 and 183.75±30.61 in test and positive control group respectively. Comparison between baseline and after 12 weeks test data were statistically significant in case of diabetic parameters regarding male test group. Table 8 shows that, among 79 female respondents of test group and 25 female respondents of positive control group, mean of fasting glucose level at baseline were 10.02±1.11and 10.18±1.92, after 1.5- month the mean was test group 8.92±0.91and positive control 8.83±1.79 and after 3 months mean was in test group 7.78±0.93 and positive control group 6.71±1.59. Comparison test data between base line and after 3 months were statistically significant. Regarding 2 hours PP glucose level, among 79 respondents of test group and 25 respondents of positive control group, mean at baseline were 16.88±2.21 and 18.70±3.88, after 1.5-month mean was in test group 10.73±1.18 and in positive control group 8.80±3.88, after 3 months mean was in test group 8.16±1.11 and positive control group 7.60±3.74. Regarding HbA1c level, the mean was 9.84±1.04 and 10.58±1.08 at baseline, 8.57±.92 and 8.38±1.08 after 1.5-month and 7.45±1.03 and 6.98±1.08 after 3 months in test and positive control group respectively. Regarding insulin level, at baseline the mean were 199.47±30.90 and 200.00±31.83. After 1.5-month they were 189.34±30.92 and 193.00±31.83 and after 3 months they were 173.47±30.90 and 188.00±31.83 in test and positive control group respectively. Comparison between baseline and after 12 weeks test data were statistically significant in case of diabetic parameters regarding female test group.

Table 1. Age distribution by treatment group according to gender

	Age	Treatment Group			
		Test group		Positive control group	
		N	(%)	N	%
Male	≤35 years	17	21.25	04	14.29
	36-45 years	28	35.00	07	25.00
	46-55 years	20	25.00	10	35.71
	56-65 years	14	17.50	06	21.43
	>65 years	01	1.25	01	3.57
	Total	80	100.0	28	100.0
Female	≤35 years	19	24.05	06	24.00
	36-45 years	34	43.04	13	52.00
	46-55 years	17	21.51	04	16.00
	56-65 years	08	10.13	02	8.00
	>65 years	01	1.27	00	0.00
	Total	79	100.0	25	100.0

Table 2. Distribution of treatment group according to Occupation

Occupation	Treatment group			
	Test group =159		Positive control group=53	
	N	%	N	%
Unemployed	03	1.9	-	-
Service holder	70	44.0	26	49.1
Agricultural work	13	8.2	02	3.8
Business	09	5.7	02	3.8
Day laborer	03	1.9	-	-
House wife	49	30.8	17	32.1
Retired	12	7.5	06	11.3
Total	159	100.0	53	100.0

Table 3. Distribution of treatment group according to presence of comorbidity

Comorbid condition	Treatment group			
	Test group =159		Positive control group=53	
	N	%	N	%
Yes	73	45.9	26	49.1
No	33	20.8	14	26.4
Don't know	53	33.3	13	24.5
Total	159	100.0	53	100.0
If yes, Name of the condition				
Hypertension	60	82.2	21	80.8
Cardiovascular disease	11	15.1	05	19.2
Hypothyroidism	02	2.7	-	-
Total	73	100.0	26	100.0

Table 4. Distribution of treatment group according to BMI (Body Mass Index)

BMI classification (Asian)	Treatment group			
	Test group		Positive control group	
	N	(%)	N	%
Underweight <18.5	-	-	-	-
Normal range 18.5-22.9	69	43.4	14	26.4
Overweight 23.0-27.5	89	56	36	67.9
Obese c ≥ 27.5	01	0.6	03	5.7
Total	159	100.0	53	100.0

Table 5. Distribution of treatment group according to family history of Diabetes

Family history	Treatment group			
	Test group =159		Positive control group=53	
	N	%	N	%
Yes	115	72.3	42	79.2
No	44	27.7	11	20.8
Total	159	100.0	53	100.0

Table 6. Distribution of treatment group according to duration of Diabetic condition

Duration of diabetes	Treatment group			
	Test group =159		Positive control group=53	
	N	%	N	%
1-5 years	94	59.1	28	52.8
6-10 years	36	22.6	11	20.8
>10 years	29	18.2	14	26.4
Total	159	100.0	53	100.0

Table 7. Distribution of treatment group according to Diabetic parameters (Male)

Diabetic Parameter Male	Test group=80	Positive Control group=28	P value of baseline and after 3 months of test group
Fasting glucose (mgdL-1)			
Baseline data	9.83±1.17	9.99±2.52	
After 1 and half month	8.61±1.07	8.98±2.52	.000
After 3 months	7.72±1.06	6.97±1.76	
2 hours PP glucose (mgdL-1)			
Baseline data	16.60±2.35	17.43±5.05	
After 1 and half month	10.00±1.17	8.29±2.42	.000
After 3 months	8.23±1.17	7.89±2.42	
HbA1c (%)			
Baseline data	9.33±1.17	10.43±2.36	
After 1 and half month	8.43±1.17	8.35±2.03	.000
After 3 months	7.45±2.03	6.87±1.18	
Insulin (p mol L-1)			
Baseline data	183.10±27.59	198.75±30.61	
After 1 and half month	175.10±27.59	190.75±30.61	.000
After 3 months	168.10±29.59	183.75±30.61	

Table 8. Distribution of treatment group according to Diabetic parameters (Female)

Diabetic Parameter Female	Test group=79	Positive Control group=25	P value of baseline and after 3 months of test group
Fasting glucose (mgdL-1)			
Baseline data	10.02±1.11	10.18±1.92	
After 1 and half month	8.92±0.91	8.83±1.79	.000
After 3 months	7.78±0.93	6.71±1.59	
2 hours PP glucose (mgdL-1)			
Baseline data	16.88±2.21	18.70±3.88	
After 1 and half month	10.73±1.18	8.80±3.88	.000
After 3 months	8.16±1.11	7.60±3.74	
HbA1c (%)			
Baseline data	9.84±1.04	10.58±1.08	
After 1 and half month	8.57±.92	8.38±1.08	.000
After 3 months	7.45±1.03	6.98±1.08	
Insulin (p mol L-1)			
Baseline data	199.47±30.90	200.00±31.83	
After 1 and half month	189.34±30.92	193.00±31.83	.000
After 3 months	173.47±30.90	188.00±31.83	

Discussion

Although type 2 diabetes is more common in those over the age of 45, the CDC reports that it is also becoming more common in younger age groups including children, teenagers, and young adults (CDC, 2023). According to the findings of the current investigation that we have conducted, the majority of diabetes patients, regardless of gender or demographic, are between the ages of 36 and 45 approximately. It has been projected that the prevalence and incidence of diabetes would both increase in the United States (Geiss et al., 2006; CDC, 2011). These projections are based on figures obtained from earlier national surveys. According to the CDC a disruption in the normal balance of glucose in the body is presently one of the leading causes of mortality in the United States (CDC, 2011). Obesity has been identified as a significant factor in the development of type 2 diabetes as well as prediabetes (Geiss et al., 2006). This is the outcome of the growing obesity epidemic that is occurring all over the world. According to the IDF, Bangladesh had 13.1 million cases of adult diabetes (20-79 years) in 2021, placing it eighth overall. Based on our projections, Bangladesh will be ranked seventh in 2045 (International Diabetes Federation, 2019). Bangladesh is one of the seven countries in the IDF SEA region. 90 million individuals in the SEA Region and 537 million people worldwide have diabetes; by 2045, this number will increase to 151.5 million (‘The International Diabetes Federation (IDF)Bangladesh, 2013.). Analysis revealed that almost 1 in 10 persons (18+) in Bangladesh had diabetes, or more than 7.9 million people by the year 2020. It should be emphasized that our data encompassed a younger demographic than the IDF estimates, hence the overall cases are exaggerated because diabetes is less common in the younger group. Nevertheless, policies supporting the implementation of diabetes prevention programs in this country are urgently needed given the high incidence of cases of diabetes in Bangladesh, which positions it among the Southeast Asian nations with the largest burden of the illness (Hossain et al., 2022). Diabetes and prediabetes affect a substantial portion of the Bangladeshi population. In 2020, more than 19 million Bangladeshis who were 18 years of age or older will have diabetes or prediabetes, according to statistics from the most current BDHS 2017–18. Diabetes was associated with age, sex, BMI, income quintile, employment status, hypertension, and the administrative division of the nation; however, neither place of residence (urban/rural) nor level of education were. These findings confirm the persistently high prevalence of diabetes and prediabetes in Bangladesh (Hossain et al., 2022). Approximately 60% of the global population utilizes traditional medicines derived from medicinal plants (Grover et al., 2002). This review centers on the utilization of Indian Herbal drugs and plants for the management of diabetes, with a particular emphasis on their application within the Indian subcontinent.

Numerous studies have shown associations between obesity, dyslipidemia, and hypertension with insulin resistance and hyperinsulinemia, two diseases that are key components of the metabolic syndrome in individuals with diabetes (Makaryus et al., 2009). Furthermore, impaired glucose homeostasis in offspring of type 2 diabetic mothers who were diagnosed at a young age has been connected to prenatal exposures and the rise in diabetes risk associated with them (Meigs et al., 2000). The best method for preventing type 2 diabetes is to be aware of its modifiable cardiometabolic risk factors (Makaryus et al., 2009). The bulk of type 2 diabetes prevalence studies, however (Nguyen et al., 2008; Srinivasan et al., 2003), only employed one baseline evaluation at middle and later years (Lyssenko et al., 2005; Wilson et al., 2007). There is a dearth of data on the correlates of type 2 diabetes' age of onset in a community among relatively young people. The current research looks at the prevalence of diabetes as people age in the Bogalusa Heart Study, a biracial (black and white) community-based examination of the development of cardiovascular disease risk commencing in infancy (Pickoff et al., 1995).

Patients with diabetes who take G. sylvestre in pill form had decreased levels of glucose in their urine (Gharpurey, 1926) reduces adrenohypophyseal activity (Gupta, 1961), as well as the hyperglycaemic response of epinephrine

(which is known to be mediated by phosphorylase and the gluconeogenetic activity), which leads to a decrease in blood sugar levels. (Gupta and Seth, 1962). It is generally known that the herb has hypoglycemic effects on people with normal diabetes Khare et al (1983), Singh et al. (2008). Shumugasundaram (1983) found that the activity of insulin-dependent enzymes such as hexokinase, glycogen synthetase, glyceraldehydes 3-phosphate dehydrogenase, and glucose 6-phosphate dehydrogenase was reduced in the diabetic tissues of rabbits, whereas the activity of insulin-independent enzymes such as glycogen phosphorylase, gluconeogenic enzymes Treatment with G. sylvestre leaves helped maintain blood glucose in the beryllium nitrate–treated rats with disturbed carbohydrate metabolism leading to liver damage (Prasad et al., 2009) and inhibited hexokinase activity in liver (Groth, 1980). In rats with diabetes caused by exposure to alloxan (Mainigi and Bresnick, 1969). In both rabbits and dogs (Prakash et al., 1986; Kar et al., 2003), the treatment led to a balance in blood glucose levels as well as serum insulin levels. This might have been caused by the repair or regeneration of the islets of Langerhans cells in the pancreas. The water-soluble extract of G. sylvestre (GS4) did not improve insulin release in normal rats when the blood sugar was maintained at 100 mg/dl; however, it did boost hormone release in diabetic rats' islets. According to Shanmugasundaram et al. (1990a), the anti-diabetic activity needed the pancreatic function that was still present. When administered to IDDM patients who were already receiving insulin treatment, the extract (GS4) decreased insulin requirements by lowering fasting blood glucose levels, glycosylated hemoglobin levels, and glycosylated plasma protein levels, while serum lipid levels virtually recovered to normal levels. (Shanmugasundaram et al., 1990a) discovered that therapy with GS4 enhanced endogenous insulin in individuals with IDDM. This was apparently accomplished by rebuilding the patients' surviving beta cells. (Shanmugasundaram et al., 1990b) In patients with type II diabetes who did not have IDDM, using the medicine GS4 for 18 to 20 months resulted in a significant reduction in plasma lipids (cholesterol, triglycerides, phospholipids, and free fatty acids). In contrast, anti-hyperglycemic drugs like sulfonylureas and biguanides work to manage blood glucose homeostasis by stimulating insulin synthesis from the pancreas (Efendi et al., 1979) and blocking gluconeogenesis (Baskaran et al., 1990), respectively. Both of these drugs are known to cause serious side effects, the most common of which is an increase in plasma levels of cholesterol, triglycerides, and free fatty acids. Additionally, the effectiveness of these medications to regulate lipid metabolism decreases over time. During a period of ten weeks, rats that were fed a high-fat diet were administered gymnema extract in order to reduce plasma triglycerides, restrict the rise in body weight, and prevent the accumulation of intraperitoneal fat and liver lipids (Shigematsu et al., 2001a, Shigematsu et al., 2001b). Activated macrophages and lymphocytes are thought to infiltrate the inflammatory focus after the first islet inflammation, which is thought to cause experimental diabetes. These cells may be where the cytotoxic oxygen radicals come from. As part of its immunomodulatory effect, shilajit has been shown to lessen macrophage and lymphocyte activation and migration. (Bhattacharya, 1995) Additionally, because it is an antioxidant, it will guard against harm from cytotoxic oxygen radicals to the pancreatic islet cell (Bhattacharya, 1995, Ghosal et al., 1995).

In one research (Trivedi et al., 2004), shilajit (100 mg/kg) administration in euglycemic rats resulted in significant hypoglycemia. According to Gupta (1966) long-term shilajit administration increases the number of pancreatic beta cells, or pancreatotrophic activity, which may lead to improved pancreatic beta cell sensitivity and quick production of a significant amount of insulin in response to hyperglycemia. Shilajit and glibenclamide together significantly reduced blood glucose levels, which is more than either medication alone did. Therefore, it is plausible that shilajit may have extrapancreatic activity in addition to its pancreatic action, which may have contributed to its hypoglycemic effect. Shilajit (100 mg/kg) has a much greater hypoglycemic impact than metformin (500 mg/kg). Shilajit alone (100 mg/kg) reduced blood sugar levels significantly, however when combined with metformin, this effect was not further enhanced. Shilajit, in all three dosages, significantly improved the lipid profile of rats with diabetes brought on by alloxan. According to reports, hyperlipidemia develops as a result of the disruption of glucose, fat, and protein metabolism that occurs with diabetes. Shilajit's advantageous effects on the lipid profile in alloxan-induced diabetic rats may be related to improved glycemic control (Austin and Hokanson, 1994; Kraus-Friedmann, 1984; Brown and Goldstein, 1983)

The lipid profile was not significantly improved by the addition of glibenclamide to shilajit (100 mg/kg) compared to shilajit alone. This might be explained by the idea that glibenclamide's improvement of the lipid profile in diabetic rats may be secondary to improved glycemic control (Chehade and Mooradian, 2000). Because glibenclamide works through a secondary mechanism, using shilajit did not result in additional improvement in the lipid profile. Combination therapy considerably ($P<0.01$) outperforms glibenclamide alone in terms of its impact on lipid profile.

Metformin improves the lipid profile primarily by reversing impaired glucose metabolism. In addition, it causes a slight drop in triglyceride levels due to a reduction in the production of very low-density lipoprotein in the liver (DeFronzo, 1995). In our investigation, a comparable finding was observed. Additionally, postprandial hyperlipoproteinemia of intestinal origin has been reported to be considerably reduced by metformin. (Chehade and Mooradian, 2000). It is hypothesized that shilajit may operate on lipid metabolic pathways via a different method than metformin because the combination of shilajit and metformin improved the lipid profile, with the exception of TG, more than either metformin or Shilajit alone.

Using alloxan-induced diabetic rats as test subjects, aqueous ethanolic solvent extracts of the stem of Bambusa Arundinaceae (Bambsaceae) were evaluated for their ability to treat diabetes. The findings indicated that aqueous ethanolic extracts had demonstrated notable protection, and that alloxan-induced diabetic rats had the greatest drop in blood glucose. According to the findings of this thorough investigation, Bambusa arundinaceaes stem shown statistically significant anti-diabetic effect when compared to the widely used glibenclamide (Macharla et al., 2012).

The DPPH test is the easiest and most reliable method for assessing the antioxidant capabilities of herbal products.The polyphenol-rich R. vesicarius (ArOH) lowers the rates of oxidation of organic materials by adding a hydrogen atom to the chain-carrying ROO* radicals (Gaurav et al., 2020). Polyphenols play a key role in the biological system's metabolism of reactive oxygen species (ROS) by preventing the generation of free radicals. According to earlier experimental and clinical investigations, it may be possible to halt the progression of diabetes by blocking carbohydrate hydrolyzing enzymes such glucosidase and amylase (Francoet al., 2020). For both human and animal species, starch serves as the main source of carbohydrates. Salivary and pancreatic -amylase randomly converts the starch into simple saccharides, resulting in smaller molecules like glucose that are absorbed into the bloodstream. According to Gaurav et al. (2020), the -amylase and -glycosidase inhibitors either stop the digestive tract from absorbing sugar or postpone the breakdown of starch into simpler sugars. Our results demonstrated that R. vesicarius might delay the breakdown of carbohydrates.

Dry extracts of Gymnema (*Gymnema sylvestre*), Bamboo Manna (*Bambusa bambos*), Bladder Dock (*Rumex vesicarius*), and Mineral Pitch (Shilajit) were given to research participants. But other than a basic spoken explanation regarding carbohydrates and sugary foods, no specific diet or exercise regimen was suggested. When assessing the study's findings, this constraint must be considered.

Conclusions

In conclusion, those with hyperglycemia have increased glucose levels, showed a positive effect and reduced blood glucose level after taking a combination of dry extracts from the following plants: Gymnema (*Gymnema sylvestre)*, Bamboo Manna (*Bambusa bambos*), Bladder Dock (*Rumex vesicarius*), and Mineral Pitch (Shilajit). To determine the appropriate diet and nutritional state of patients, more study is required. Additionally, depending on how severe the diabetic's condition is, the effects of a combination of dry extracts from the following plants may be used: Gymnema (*Gymnema sylvestre*), Bamboo Manna (*Bambusa bambos*), Bladder Dock (*Rumex vesicarius*), and Mineral Pitch (Shilajit).

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The Effect of Different Cooking Methods and Addition of Different Sweeteners on the Physicochemical and Antioxidant Properties of Aronia Marmalade

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ABSTRACT

The present study examined the physicochemical properties, antioxidant activity (DPPH, ABTS, and FRAP), and sensory properties of aronia marmalades prepared with different cooking methods (CM) (boiled (B) and pressure-boiled (PB)) by adding sugar (S) and stevia prebiotic fiber sweetener (SP). Ash, reducing sugar, sucrose, viscosity, L*, a*, b*, C*, and H° values, and total sugar content of aronia pulp and marmalades differed significantly by cooking method and sweetener type (ST). Hydroxymethylfurfural could not be detected in aronia pulp and marmalades. Concerning CM, TPC (total phenolic content) and TMA (total monomeric anthocyanin) values were found to be significantly higher in PB cooking than in the B cooking method. On the other hand, TFC (total flavanoid content) was statistically higher in boiled marmalades. According to CM, the DPPH antioxidant activity of marmalades was significantly higher in B marmalades. The TPC, TMA, TFC, and antioxidant properties of marmalades differed sigficantly by ST. The TPC of marmalades prepared with SP addition was higher than that of S-added marmalades and control. According to ST, whereas the antioxidant activities (DPPH, ABTS, and FRAP) of S and SP-added marmalades were lower compared to the control, the antioxidant activities determined by DPPH and ABTS among S and SP-added marmalades were higher in SP-added marmalades. The panelists gave the highest scores to BSC (boiled S-added marmalade). Considering the overall acceptance scores, the second highest score was given to BST (boiled SP-added marmalade). In other words, in terms of sensory evaluation, boiled marmalades received higher overall acceptance scores, while PBST (PB SP-added marmalade) received the lowest scores. According to these results, astringency components decrease with cooking in an open vessel. Furthermore, it can be said that sugar masks this astringent taste.

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Introduction

Aronia berry, which belongs to the Maloideae subfamily of the *Rosaceae* family and is also known as chokeberry, is widely cultivated worldwide, particularly in Europe and North America (Kokotkiewicz et al., 2010; Huang et al., 2022). Two species of the *Aronia* genus can be distinguished: *Aronia melanocarpa (Michx.) Elliot*, known as black chokeberry, and *Aronia arbutifolia* (L.) Pers. (red chokeberry). The third type is a hybrid of the two mentioned species, *Aronia prunifolia*, purple chokeberry (Jurendić and Ščetar, 2021). Native Americans traditionally used the berries of *A. melanocarpa* to cure the common cold. Nowadays, it is mainly used as an ornamental plant and in the production of fruit juice, jam, liqueurs, and wine (Kokotkiewicz et al., 2010). Aronia (*Aronia melanocarpa*) berries are typically deep purple and have high anthocyanin contents and health-promoting properties such as antioxidant, anti-inflammatory, and antibacterial activity (Jang and Koh, 2023). The polyphenol profile of aronia berries is well-structured, with cyanidin glycosides (cyanidin-3-*O*-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and cyanidin-3-*O*-xyloside) as the main anthocyanins. The anthocyanin content of chokeberry (737 mg/100 g FW) is considerably higher compared to other anthocyanin-rich berries (such as blueberry, strawberry, and raspberry) (Gao et al., 2022). It contains highly polymerized B-type proanthocyanidins, chlorogenic acids, and quercetin glycosides. Aronia berries contain high amounts of anthocyanin and proanthocyanidin polyphenols that may help reduce the risk of cardiovascular, and gastrointestinal diseases,

diabetes, and cancer. However, aronia berries have an astringent and bitter taste. Hence, their appeal to consumers is primarily due to their bioactive compounds and health benefits. (King et al., 2021; Jurendić and Ščetar, 2021).

Fruits and vegetables are perishable, and their fresh consumption is seasonal. Therefore, they need to be processed with different preservation techniques, such as jam and marmalade making. Accordingly, processing aronia berries into marmalademay be a good alternative to meet these need, apart from their fresh consumption. In the Turkish Food Codex Communiqué on Jam, Jelly, Marmalade and Sweetened Chestnut Puree, "traditional marmalade" is defined as "a mixture of fruit pulp, puree, fruit juice, and aqueous extracts or edible parts of plants, such as roots, leaves, and flowers brought to a spreadable consistency by adding sugars and water when necessary" (Anonymous, 2006). Cooking in an open vessel or under vacuum can be applied to make products such as jam and marmalade spreadable. It is reported that since a lower temperature is applied in vacuum cooking than in open cooking, color and aroma properties will improve, and nutrient loss will decrease (Özdemir et al., 1998). Moreover,due to increased rates of diabetes and obesity, today's consumers gravitate to foods prepared from raw materials that are low in calories, appealing to the palate, mostly grown naturally, and have health benefits.

There is no study in the literature on marmalade production with aronia berries using different sweeteners and different cooking methods. Hence, it was aimed to determine changes in various physicochemical and antioxidant properties of aronia berries during their processing into marmalade using different sweeteners and different cooking methods. Furthermore, finding a practical way to reduce the astringencyof products produced with aronia berries is among the most important points to open the aronia industry to the market. Therefore,panelist evaluations, aimed to determine the impacts of different sweeteners on the astringent taste and overall acceptance during the processing of aronia into marmalade to reduce astringency.

Materials and Methods

Material

The aronia marmalades used in the study were produced with fresh aronia (*Aronia melanocarpa*) berries grown in Kırklareli, Vize, Türkiye. Crystal white granulated sugar (S) and stevia prebiotic fiber sweetener (SP) (Fibrelle, Türkiye) used in marmalade production were purchased from a local market in Erzurum.

Marmalade Production

Aronia marmalade formulation was determined by preliminary trials. During marmalade preparation, 400 ml of water was added to 400 g of aronia berries, whose stems were separated and removed, and they were crushed homogeneously for 5 minutes with a laboratory-type grinder (Waring HGB2WTS3, USA). Then sweeteners (sucrose (S) and stevia prebiotic fiber sweetener (SP)) were added separately at the rate of 40%, and marmalade was cooked under vacuum with an evaporator (Heidolph Laborota 4000 Efficient, Germany), as described by Korus et al. (2015). In the study, aronia pulps prepared without adding sweeteners were accepted as a control. It was prepared by first mixing 2.35 g pectin with water, and its mixing was ensured by adding it toward the end of cooking. In both cooking methods (boiled (B) and pressure-boiled (PB)), cooking was stopped when the water-soluble dry matter content of marmalades measured with a refractometer (Abbe Zeiss, Germany) was 55-60% and the pH measured with a pH meter (Ohaus, starter 3100, USA), as specified by Kaya et al. (2016), was between 2.8 and 3.5. Before the end of heat treatment, 10 g of citric acid was added and boiled for another 1-2 minutes. The hot marmalade was placed in glass jars with sealed twist-off lids as, then turned upside down for a while and left overnight. Afterward, the jars were brought to a straight position and stored at room temperature and in a dark place until the analysis.

Marmalades were coded as follows: BSC: Boiled sucrose-added marmalade, PBSC: pressure-boiled sucrose-added marmalade, BST: Boiled stevia prebiotic fiber-added marmalade, PBST: Pressure-boiled stevia prebiotic fiber-added marmalade.

Determination of Dry Matter

3-5 g samples taken from the homogenized aronia marmalade were kept in an oven at 100-105 °C until they reached a constant weight. After the samples were cooled in a desiccator, they were weighed. The total dry matter value was determined as a percentage using the values obtained (Cemeroğlu, 2013).

Ash Determination

After the moisture in the homogenized marmalade samples (3.0±0.1 g) was evaporated in the oven, a few drops of ethyl alcohol were dripped onto the samples, and they were burned in the muffle furnace (Karl Kolb M011, Gerhardt, Germany) at 550±25 °C until white ash was formed. After the crucibles were cooled in the desiccator as a result of combustion, the remaining ash was weighed, and its amount was determined in g/100 g (Cemeroğlu, 2013).

Determination of Total Sugar, Reducing Sugar, and Sucrose

The Lane-Eynon volumetric method was employed to determine sucrose, reducing sugar, and total sugar contents of the marmalade samples. The samples were analyzed before and after inversion. For the inversion procedure, 5 ml of 37% HCl was added to 50 ml of clear filtrate taken into a 250 ml volumetric flask, the temperature was brought to 67 °C in a water bath, and the samples were kept for 5 minutes. Afterward, phenolphthalein was added to the cooled flask and titrated with 4 N NaOH, and titration was terminated when the solution color became light pink. The titrated solution was completed to 250 ml with distilled water, the analysis was continued, and the amounts spent in the end were recorded. Calculations were performed with the recorded pre- and post-inversion amounts, and the total sugar, reducing sugar, and sucrose contents were determined (Cemeroğlu, 2013).

Hydroxymethyl Furfural Analysis

When determining hydroxymethyl furfural (HMF) content, first, 5 g of the sample was weighed and dissolved

in 10 ml of distilled water. Two ml of the prepared solution was taken, and 5 ml of p-toluidine solution was added to it. One ml of barbituric acid solution was added to one of the two test tubes prepared in this way, and 1 ml of distilled water was added to the other, and the contents were mixed by vortexing. The absorbance value of the solution was determined by reading in the spectrophotometer at a 550 nm wavelength. The HMF content was calculated using the formula below (Cemeroğlu 2013):

HMF (mg/kg) = A x 162

A: Absorbance value

Viscosity determination

The viscosity values of marmalade samples were measured in the range of 0.3-10,000 mPa.s at 20 °C using an SV-10 Viscometer (A&D Company, Japan). The results were determined in mPa.s (Wang et al., 2016).

Color determination

The color values of marmalade samples were measured with a colorimeter (Konica Minolta CR-400, Korea). The samples' color intensities were determined with a colorimeter making three-dimensional measurements in the CIE (L*, a*, b*, C*, H°) system. H° (Hue angle), L* (bright: 100, dark: 0), C* (Chroma, color saturation), a* (red: +60, green: -60), and b* (yellow: + 60, blue: -60) values were determined as a result of the readings, and all readings were performed on a white background at 20±2°C (Zor and Şengül, 2022).

Total Phenolic Content (TPC) Analysis

While preparing the aronia marmalade extracts to be used throughout the study, 3 grams of each of the marmalade samples were weighed for extraction, and 30 ml of methanol was added to them. They were kept in an ultrasonic water bath for 45 minutes, and this procedure was repeated 3 times at 15-minute intervals. Afterward, the extracts taken into the tubes were centrifuged at 6000 rpm at 4 °C for 15 minutes and filtered through Whatman No 2 filter paper. The acquired filtrates were used for total phenolic content (TPC), total monomeric anthocyanin (TMA) content, total flavonoid content (TFC), and antioxidant activity analyzes (Zor et al., 2022).

To the total phenolic content in the marmalade samples, 100 µl of the extracts were taken, 0.2 N 2.5 ml FCR (Folin-Ciocalteau reagent) was added to them, and they were left for 3 minutes. After the waiting period, 2 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture and incubated for 2 hours. At the end of the period, the samples' absorbance values were read in a spectrophotometer (PG Instruments T60V, UK) at a 760 nm wavelength. To determine the samples' phenolic content, the equation acquired from the graph prepared using the gallic acid standard was used, and the total phenolic content was calculated as gallic acid equivalent (mg GAE/g) (Meda et al., 2005).

Determination of Total Monomeric Anthocyanin (TMA) Content

The monomeric anthocyanin (TMA) content of marmalade was determined according to the pH differential method. The anthocyanin concentration was calculated with the difference of the measured absorbance values when the ambient pH was 1.0 and 4.5. The principle of this method is based on the fact that samples are in the form of colored oxonium when the ambient pH is 1.0 and samples are in the form of colorless carbinol pseudobase when the ambient pH is 4.5 (Cemeroğlu, 2013). The sample extracts were diluted with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5), and absorbance was measured with a spectrophotometer (PG Instruments T60V, UK) at 515 and 700 nm wavelengths after 30 minutes. The equation below was used when calculating the total monomeric anthocyanin content (Sun et al., 2009).

A=(A510-A700) pH 1.0-(A510-A700) pH 4.5

A: Absorbance difference

A515: absorbance value at 515 nm

A700: absorbance value at 700 nm

The formula used to calculate the monomeric anthocyanin content as cyanidin-3-glucoside equivalents is as follows:

TMA (mg/l)=A(MA)(Sf)1000/(ε)ʃ

MA=449.2 (molecular weight of cyanidin 3-glucoside)

Sf=Dilution factor,

ε=Molar absorptivity

ʃ=Layer thickness of the cuvette used in the spectrophotometer (ʃ=1 cm)

***Total Flavonoid Content* (TFC)**

Total flavonoid content was determined spectrophotometrically according to the method suggested by Koçak et al. (2018). First, 0.25 ml of the sample extracts were taken and 1.25 ml of distilled water was added to it. Then, 0.075 mL of 0.05g/mL $NaNO_2$ was added and incubated for 6 minutes after vortexing. At the end of the period, 0.15 mL of 0.1 g/mL $AlCl_3 \cdot 6H_2O$ was added and vortexed, then left for 5 minutes. Finally, 0.5 mL of 1 mol/L NaOH was added, mixed and then incubated for 15 minutes. At the end of the period, the samples' absorbance values were read in the UV-visible spectrophotometer (PG Instruments T60V) at a wavelength of 510 nm. In the calculations, the equation obtained from the calibration curve drawn as a result of the measurements made by preparing 10-250 mg/L quercetin was used. The total flavonoid content is given as quercetin equivalent (QE)/g.

Determination of Antioxidant Activity

Determination of Antioxidant Activity with the DPPH Method

To analyze DPPH radical scavenging activity in marmalade samples, 10, 20, and 30 µg/ml were taken from the sample extracts and then made up to 2 ml with ethanol. 500 µl of DPPH solution was added to the samples, completed to 2 ml with ethanol, mixed homogeneously by vortexing (Heidolph Reax Top, D-91126 Schwabach, Germany) and incubated for 30 minutes in a dark environment at room temperature. At the end of the incubation period, the samples' absorbance was read in the spectrophotometer at a 517 nm wavelength (Popović et al., 2012). The %inhibition (%I) of the extracts was calculated according to the formula using the absorbance values:

$\%I = ((A_c - A) \times 100) / A_c$

(A_c: Absorbance of the control, A: Absorbance of the extract)

IC_{50} values were calculated from the equation acquired using percentage inhibition values versus different concentrations of the samples.

Determination of Antioxidant Activity with the ABTS Method

ABTS (2.2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) solution to be used for $ABTS^{\bullet+}$ radical scavenging activity analysis in marmalade samples was prepared by adding potassium persulfate solution and mixing it for 16 hours in the dark environment. The absorbance value of the prepared solution was measured at a wavelength of 734 nm in the spectrophotometer and diluted to 0.700±0.025. 10 µl of the sample extracts were taken and 990 µl of ABTS radical was added to them. Then they were mixed homogeneously by vortexing and incubated for 6 minutes in a dark environment. The samples' absorbance values were read at a wavelength of 734 nm at the end of the incubation period (Özkan et al., 2007). The %inhibition (%I) of the extracts was calculated according to the following formula using the absorbance values:

$\%I = (A_c - A) \times 100/A_c$

(A_c: Absorbance of the control, A: Absorbance of the extract)

IC_{50} values were calculated from the equation acquired using percentage inhibition values versus different concentrations of the samples.

Determination of Antioxidant Activity with the FRAP Method

It is based on reducing Fe^{3+} ions in the Fe $(TPTZ)^{3+}$ mixture present in the radical to be used in the determination of antioxidant activity in tea samples with the FRAP method to the blue-colored Fe $(TPTZ)^{2+}$ complex in the acidic medium (Koçak et al., 2018). Antioxidant activity was determined withthe FRAP method by making some changes to the method reported by Koçak et al. (2018). The solvents used in the study were prepared as follows;

1-Three solutions were prepared daily as acetate buffer (pH 3.6) by dissolving 3.1 g sodium acetate+16 mL acetic acid in 1 L solution,

2-By dissolving 0.156 g of TPTZ (2,4,6-tripyridyl-s-triazine) in 50 mL of ethanol, and

3-As 0.5404 g $FeCl_3 \cdot 6H_2O$+2 mL HCl (37% m/m) in 100 mL solution.

After the solutions were prepared, 80 mL of the 1st solution, 8 mL of the 2nd solution, and 8 mL of the 3rd solution were taken and mixed. In this way, the FRAP reagent was prepared . 0.9 mL of FRAP reagent was added to 0.1 mL of the sample extract, and the mixture was vortexed. After 4 minutes, the absorbance was measured at 593 nm. A calibration curve was acquired with 5-25 micromole solutions of Trolox prepared with methanol. The results from the calibration curve were calculated as µM Trolox equivalent (TE)/g.

Sensory Analysis

The panelist group, consisting of students and faculty members at the Department of Food Engineering of Atatürk University (Erzurum, Turkey), carried out sensory tests at the Department of Food Engineering at Atatürk University. The panelists evaluated various parameters of aronia marmalade samples (appearance, color, odor, texture, taste, fluidity, and overall acceptance). Marmalades, randomly named with three digit numbers, were presented to each panelist at room temperature in glass plates, allowing them to determine the product features clearly. Semi-expert graduate students and academicians at Atatürk University, Department of Food Engineering, conducted sensory analysis.

Statistical Analysis

The data obtained in triplicate were analyzed with the SPSS 20.0 program. The results were expressed as mean values with standard deviation (±SD). One-way analysis of variance (ANOVA) was carried out to determine the significant group differences between the means ($p \leq 0.05$, $p \leq 0.01$). Duncan's multiple range test was used to compare mean values. Moreover, principal component analysis (PCA) was applied to some data to facilitate the identification of similarities and differences between the samples (SIMCA-P + 14.1, UMETRICS).

Results and Discussion

Table 1 contains some physical and chemical properties of aronia marmalades produced withdifferent sweeteners (S, and SP) and different cooking methods (boiled, and pressure-boiled). Considering the cooking method, dry matter content differed insignificantly ($p>0.05$), while the ash content of pressure-boiled marmalade was significantly higher ($p<0.05$). As seen in Table 1, the dry matter content of S-added marmalades was significantly ($p<0.05$) higher than that of SP-added marmalades and the control, whereas ash content was statistically lower in sweetener-added marmalades. This situation is thought to have been caused by the fact that the fruit ratio in the control and the fruit ratio in marmalades were not equal according to the formulation. Kaya et al. (2019) reported that the dry matter content of the marmalade in which a mixture of sugar and stevia Reb D was used among hawthorn marmalades produced by adding different sweeteners was higher than marmalades prepared by adding sugar alone. The same study stated that ash content was the lowest in hawthorn marmalades with the addition of commercial stevia whereasthe highest ash content was in hawthorn marmalades prepared with stevia Reb D.

It is known that some amount of sucrose in jam and marmalade production undergoes inversion depending on cooking temperature and time (Özdemir et al., 1998). In the study, the reducing sugar content of pulp and marmalades cooked by the pressure-boiled method was higher compared to boiled marmalades. It can be said that less Maillard reaction occurs in pressure-boiled marmalades due to low temperature, and therefore, thereducing sugar content is higher.

The reducing sugar, sucrose, and total sugar contents of marmalades differed significantly by sweetener type ($p<0.05$). The reducing sugar and total sugar contents of SP-added marmalades were lower compared tothe control and S-added marmalades. The lowest sucrose content was found in the control. It is thought that the total sugar detected in SP-added marmalades is the sugar passing from fruit to marmalade.

Table 1. Some physicochemical properties of marmalades according to cooking method and sweetener type

	DM	A	RS	S	TS	HMF
Cooking method (CM)						
Boiled	55.85±18.15	0.78±0.20^{b}	12.41±3.32^{b}	11.53±16.16^{a}	23.94±17.96^{b}	ND
Pressure-boiled	56.61±17.87	0.82±0.19^{a}	28.22±21.85^{a}	1.12±0.94^{b}	29.34±22.77^{a}	ND
Significance	ns	**	**	**	**	-
Sweetener type (ST)						
Control	32.54±0.70^{c}	1.06±0.02^{a}	14.95±0.35^{b}	0.32±0.08^{c}	15.26±0.41^{b}	ND
Sucrose	71.36±1.41^{a}	0.65±0.02^{c}	35.95±23.39^{a}	17.70±16.83^{a}	53.65±6.64^{a}	ND
Stevia prebiotic fiber sweetener	64.79±0.62^{b}	0.69±0.06^{b}	10.06±2.26^{c}	0.95±0.36^{b}	11.01±1.93^{c}	ND
Significance	**	**	**	**	**	-
CMXST	ns	**	**	**	**	-

	V	L*	a*	b*	C*	H°
Cooking method (CM)						
Boiled	7.72±1.57^{a}	26.61±0.70^{a}	2.17±1.35^{a}	2.30±0.23^{a}	3.25±1.13^{a}	50.70±13.14^{b}
Pressure-boiled	5.50±2.11^{b}	26.46±0.43^{b}	1.78±1.12^{b}	2.28±0.21^{b}	2.97±0.88^{b}	55.39±13.11^{a}
Significance	**	*	**	*	**	**
Sweetener type (ST)						
Control	5.93±2.85^{c}	27.25±0.35^{a}	3.62±0.40^{a}	2.58±0.04^{a}	4.45±0.34^{a}	35.59±2.67^{b}
Sucrose	7.01±2.16^{a}	26.06±0.08^{c}	1.14±0.09^{b}	2.16±0.04^{b}	2.45±0.02^{b}	62.17±2.36^{a}
Stevia prebiotic fiber sweetener	6.89±1.36^{b}	26.29±0.07^{b}	1.17±0.17^{b}	2.13±0.05^{c}	2.43±0.12^{b}	61.38±3.09^{a}
Significance	**	**	**	**	**	**
CMXST	**	**	**	**	**	**

DM: Dry Matter (g/100 g); A: Ash (g/100 g); RS: Reducing Sugar (g/100g); S: Sucrose (g/100g); TS: Total Sugar (g/100g); HMF: HMF (mg/kg); V: Viscosity (mPa.s); Note: $^{a-c}$Means with different letters in the same column are significantly different (*p < 0.05. **p < 0.01); Sign: Significance; ns: not significant (p > 0.05), ND: Not detected

Table 2. TPC, TMA, TFC and antioxidant properties of marmalades

	TPC	TMA	TFC	DPPH	ABTS	FRAP
Cooking method (CM)						
Boiled	10.43±1.79^{b}	2217.52±941.74^{b}	31.71±6.93^{a}	364.51±61.90^{b}	192.43±32.00	51.35±13.90
Pressure-boiled	11.93±2.51^{a}	4581.17±1267.34^{a}	29.00±6.37^{b}	431.08±90.95^{a}	192.70±37.41	51.48±12.93
Significance	**	**	**	**	ns	ns
Sweetener type (ST)						
Control	11.35±1.60^{b}	4792.72±1456.78^{a}	37.39±4.10^{a}	324.96±40.49^{c}	157.81±7.14^{c}	68.46±2.03^{a}
Sucrose	9.84±1.03^{c}	2424.98±872.60^{c}	24.08±3.78^{c}	487.93±71.13^{a}	222.78±21.61^{a}	43.75±4.67^{b}
Stevia prebiotic fiber sweetener	12.34±3.13^{a}	2980.33±1566.41^{b}	29.58±3.14^{b}	380.50±13.49^{b}	197.11±28.11^{b}	42.03±5.07^{b}
Significance	**	**	**	**	**	**
CMXST	**	**	**	ns	**	**

TPC: TPC (mg GAE/g); TMA: TMA (mg Cy-3-Gl/kg); TFC: TFC (mg QE/g); DPPH: DPPH (IC_{50} µg/ml); ABTS: $ABTS^{\cdot+}$ (IC_{50} µg/ml); FRAP: FRAP (mM TE/100 g); Note: $^{a-c}$Means with different letters in the same column are significantly different (*p < 0.05. **p < 0.01); Sign: Significance; ns: not significant (p > 0.05

The formation of HMF, which is not present naturally in fruits but is an intermediate product of the Maillard reaction, varies depending on the temperature and duration of heat treatment applied during production and storage and various factors such as pH, dry matter, type and concentration of the reacting compounds (Şengül et al., 2018). HMF could not be detected in aronia pulp and marmalades (Table 1), showing that an appropriate procedure was followed in marmalade production. It may also thought that it may have originated from the absence of amino acids in the compound, required in the HMF formation process.

Concerning the viscosity values according to the cooking method, it was found that boiled marmalades had higher viscosity. The use of SP in marmalades significantly increased viscosity compared to the control group (p<0.05). In the study by Öztürk (2023), the panelists evaluating the sensory properties of low-sugar orange marmalade using Reb A stated that the consistency of the samples containing only Reb A was thin in comparison with the standard sample. Therefore, it is thought that the use of stevia prebiotic fiber sweetener in our study increased in viscosity due to the higher water-holding capacity because of the fibrous components in the formulation of SP.

L*, a*, b*, and C* values were statistically significantly higher in boiled marmalades in terms of the cooking method and in the control in terms of the sweetener type than in other marmalades. Since the L* value represents the lightness or darkness of the product color, it is important to determine the quality characteristics. Upon examiningsweetener-added marmalades, it was seen that SP-added marmalades had higher L* value than S-added marmalades. Likewise, Suna et al. (2023) reported that sweetener-added persimmon marmalades had higher L* values than sugar-added marmalades. The a*, b*, and C* values of boiled pulp and marmalades were higher than the values of pressure-boiled samples. Which may be explained by the possible Maillard and non-enzymatic browning reactions during heat

treatment. Accordingly, red pigments may be reduced due to the degradation of the compounds causing the color. The addition of S and SP significantly ($p<0.05$) reduced a*, b*, and C* values (Table 1). The C* value shows the color tone of products, and its values are low in pale colors and high in vivid colors (Öztürk, 2023). It is thought that this decrease in the color values of S and SP-added marmalades compared to the control originates from formulation and heat treatment.

Contrary decreased C* values, H° values, which are important in defining the color of marmalades, were statistically higher in pressure-boiled marmalades. Furthermore, according to the sweetener type, the H° value was similar in sugar and stevia prebiotic fiber sweetener-added marmalades and significantly higher than the control ($p<0.05$). Likewise, Suna et al. (2023) reported that the addition of sweetener in persimmon marmaladesincreased hue values.

Table 2 shows changes in the TPC, TMA, TFC, and antioxidant properties of aronia marmalades by the cooking method and sweetener type. The TPC and TMA content of pressure-boiled samples were significantly higher than those of boiled ones ($p<0.05$). On the other hand, TFC was statistically higher in boiled marmalades. Scibisz and Mitek (2009) reported that the thermal degradation of anthocyanins was a first-order reaction and high temperature increased the adverse effects on anthocyanins in the presence of oxygen and fructose. Moreover, it is reported that nutrient loss can be reduced by cooking products, such as jam and marmalade, under vacuum (Özdemir et al., 1998).

The DPPH antioxidant activity of boiled marmalades was significantly higher ($p<0.05$). However, it was concluded that the effect of the cooking method on the antioxidant activities determined by ABTS and FRAP was insignificant ($p>0.05$).

The TPC, TMA, TFC and antioxidant properties of marmalades differed significantly by sweetener type ($p<0.05$) (Table 2). The TPC of SP-added marmalades washigher than S-added marmalades and control (Figure 1). Similar to our study, studies have reported that the addition of stevia to various marmalades increases TPC (Kaya et al., 2019; Suna et al., 2023). Contrary to our results, Kamiloglu et al. (2015) stated that the use of sweeteners instead of sugar in jams and marmalades, in general, did not cause a significant difference in total phenolic content. Scibisz and Mitek (2009) indicated that blueberry jam with high sugar content had higher TPC compared to jams with low sugar and sweetener (Aspartame and Acesulfame-K) addition.

The TMA and TFC values were higher in the control sample. Additionally, the TMA and TFC of SP-added marmalades were higher in comparison with S-added marmalades (Figure 1). It is thought that one of the reasons for the higher TMA and TFC in the control group is that the added sweeteners reduce the amount of fruits relatively; thus, the TMA and TFC from the fruit decrease. Contrary to the results obtained from our study, Scibisz and Mitek (2009) found that TMA content was higher in sugar-sweetened jams than in sweetener-added jams (Aspartame and Acesulfame-K).

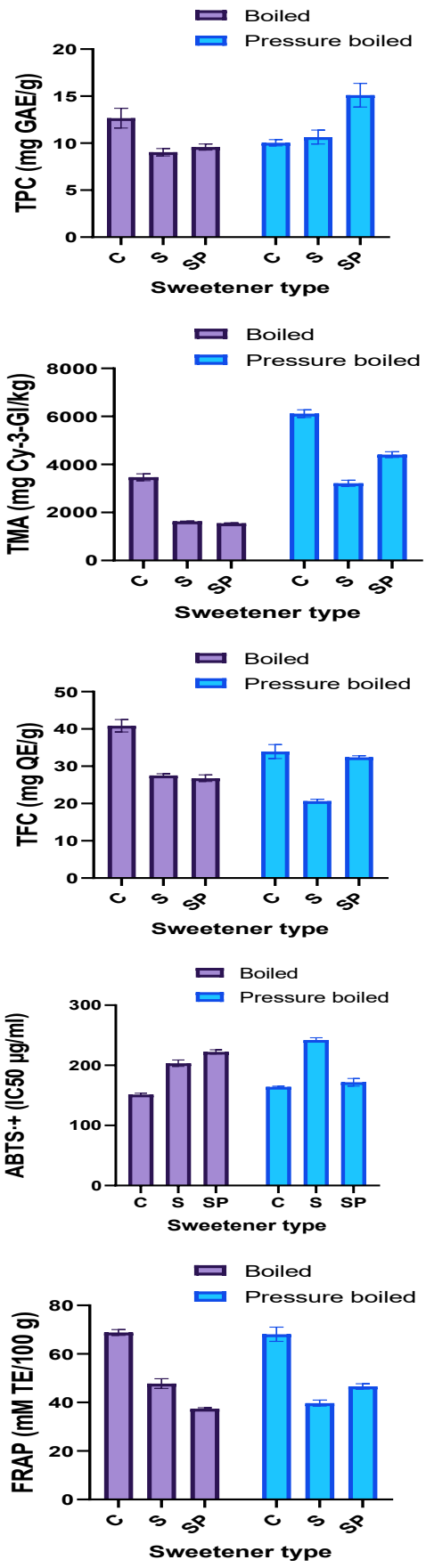


Figure 1. The effect of cooking method x sweetener type interaction on the TPC, TMA, TFC and antioxidant properties of aronia pulp and marmalades

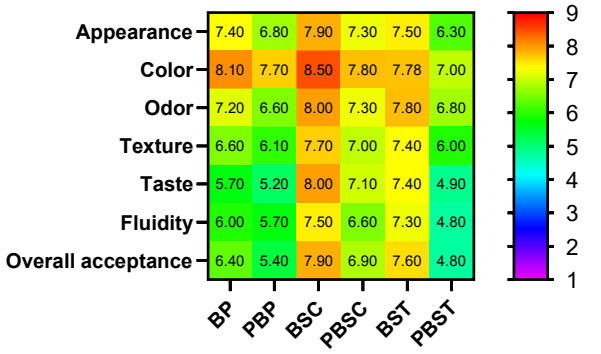
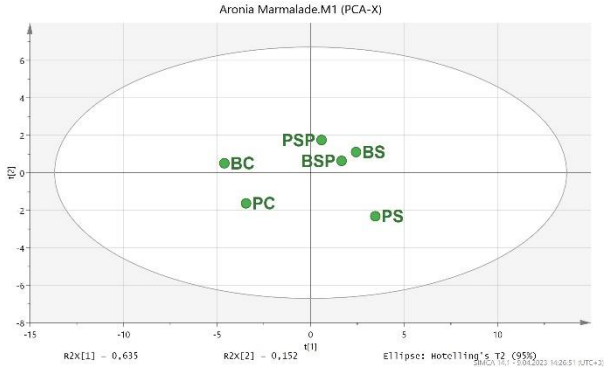
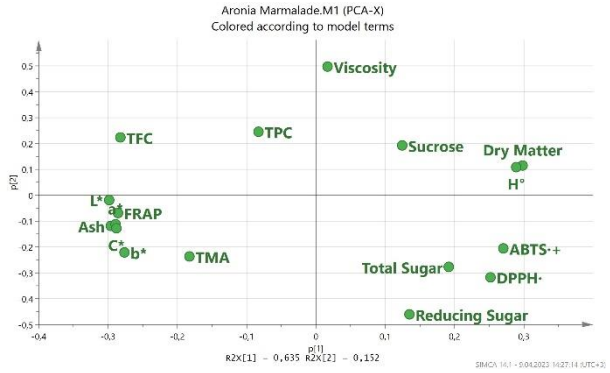


Figure 2. Sensory properties of aronia pulp and marmalades.

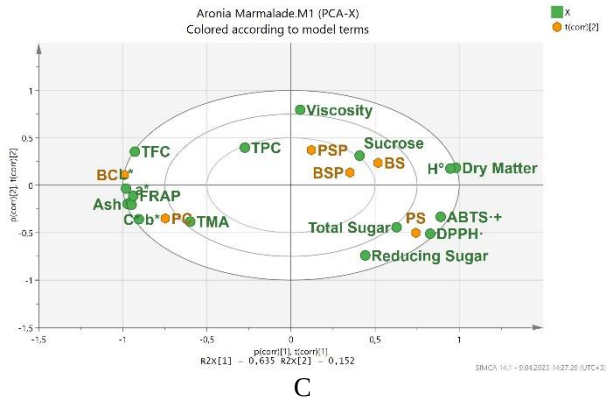
In the figure; BP: Boiled aronia pulp, PBP: Pressure-boiled aronia pulp, BSC: Boiled sucrose-added marmalade, PBSC: pressure-boiled sucrose-added marmalade, BST: Boiled stevia prebiotic fiber-added marmalade, PBST: Pressure-boiled stevia prebiotic fiber-added marmalade.



A



B



C

Figure 3. Score scatter plot (A), loading scatter plot (B), and biplot (C) of the principal component analysis (PCA) (PC1 vs. PC2) for the attributes in marmalade

Whereas the antioxidant activities (DPPH, ABTS, and FRAP) of S and SP-added marmalades were be lower than those of the control, DPPH and ABTS antioxidant activities among S and SP-added marmalades were higher in SP-added marmalades (Table 2, Figure 1).

At this point, the knowledge that carotenoids and phenolics with antioxidant and antimicrobial properties in stevia act as natural substances supporting the antioxidant capacity of the food to which it is added supports our results (Shivanna et al., 2013; Suna et al., 2023). Similar to our results, Kaya et al. (2019) reported a decrease in the antioxidant capacity of hawthorn marmalades determined by ABTS compared to pulp. Cıngoz and Demırdoven (2022) also stated that the addition of stevia and spices increased the total phenolic content and antioxidant capacity values of marmalades. In our study, the FRAP antioxidant activity analysis determined that the antioxidant activity of the control was the highest and the antioxidant activities of S and SP-added marmalades were statistically similar. Kamiloglu et al. (2015) reported that the addition of sugar or sweetener (containing sorbitol and saccharin) to black carrot marmalades caused similar antioxidant activities determined by ABTS and CUPRAC.

In the sensory evaluation of aronia pulp and marmalades, the panelists gave the highest scores to BSC marmalade in terms of appearance, color, odor, texture, taste, fluidity, and overall acceptance. Considering the overall acceptance scores, the second highest score was given to BST marmalade (Figure 2). In other words, in terms of sensory evaluation, boiled marmalades received higher overall acceptance scores, while PBST received the lowest scores. According to these results, astringency components decreased with cooking in an open vessel. Furthermore, it can be said that sugar masks this astringent taste.

Cıngoz and Demırdoven (2022) reported that, according to the sensory evaluation results of pumpkin marmalades prepared with four different recipes by adding spices, stevia, and granulated sugar in different proportions, granulated sugar-added pumpkin marmalades without spices received the highest scores, followed by stevia-added samples. In their study on the effect of usingcarob flour, stevia and cinnamon in the production of diabetic strawberry jam on the product's sensory properties, Mutlu et al. (2021) reported that the control group (industrially produced traditional strawberry jam) had the highest average values according to the general acceptability scores. They also reported that the traditional strawberry jam produced with stevia + cinnamon had the closest mean values to the control group in terms of all parameters. Öztürk (2023) indicated that for low-sugar orange marmalade using Rebaudioside A, panelists stated that they could purchase marmalades produced with Reb-A when on a diet, but they liked the recipes produced with Reb A-sugar mixture more.

Principal component analysis (PCA)

Principal component analysis (PCA) was performed to determine differences between samples by evaluating some physicochemical properties and antioxidant activities, total phenolic, total flavonoid, and total monomeric anthocyanin contents of aronia marmalades prepared withdifferent sweeteners and cooking methods.

Figures 3A-C show the score scatter plot of the marmalade samples, the loading scatter plot, and the two plots of the principal component analysis. The first two principal components (PC1=63.50% and PC2=15.20%) explained 78.70% of the variance.

As a result of the analysis, marmalade samples could be divided into two main groups (Figure 3A). While samples in the control group (BC, PC) were located on the right side of PC1, sweetener-added samples cooked in an open vessel and under pressure were located on the left side of PC1 (Figure 3A). TFC FRAP, TMA, L*, a*, b*, and C* values were located closely to the control samples (Figure 3C), showing that these characteristics of the control samples were higher. Furthermore, the antioxidant activity determined with the DPPH and ABTS methods in marmalade (PS) cooked under vacuum and added with sucrose was in a close position with the IC_{50} values. Since it is known that the IC_{50} value and antioxidant activity are inversely correlated, we can say that the lowest antioxidant activity was in this sample (PS) (Figure 3C). The said results showed that the total ash content, antioxidant activity, TPC, TFC, and TMA contents were the highest in the controlsamples, followed by stevia-added samples cooked under vacuum.

Conclusion

In line with all these results, it was seen thatmarmalade productionunder vacuum with stevia prebiotic fiber sweetener was suitable in terms of preserving the product's nutritional components. The TPC and TMA content of the samples cooked under vacuum were higher than the samples cooked in an open vessel, and the antioxidant activity determined with the ABTS and FRAP methods did not differ statistically significantly. When evaluated in terms of sweetener addition, it was determined that the TPC, TMA, TFC and antioxidant activity determined with the DPPH, ABTS, and FRAP methods of stevia prebiotic fiber sweetener-added marmalades were higher than those of sucrose-added samples. When the cooking method and sweetener parameters were evaluated together, it was seen that cooking under vacuum and the addition of stevia prebiotic fiber sweetener preserved the product's properties better. According to the study results, the use of stevia prebiotic fiber sweetener can be recommended in products sweetened with sugar, such as marmalade.

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Molecular Investigation of the Impact of Thermal Processing Techniques on Tropomyosin Crustacean Allergens

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ABSTRACT

While shellfish species are widely consumed due to their nutritional advantages, they are also among the top eight food items for food-borne allergies. Five distinct thermal processing techniques were applied to the crustacean to investigate the tropomyosin level variations caused by heat processing. Fresh shrimp and prawns were utilized as controls for the determination of allergen-encoding genes. Prior to molecular analysis, the proximate composition and acidity of raw and processed samples were also performed. The yield and purity of DNA were also determined. Melting curve and gel electrophoresis tests verified the existence of allergen-coding genes. Thermal processing procedures affected the proximate composition, particularly the total protein and fat concentrations, according to the findings. Following the heat treatment, the pH levels decreased, particularly in the grilled samples. There were also significant differences in the quantity and quality of the extracted DNA. Regardless of crustacean species, the tropomyosin-encoding gene was detected in both fried and grilled samples. These findings demonstrated that RT-PCR identification and validation of the crustacean allergy gene by gel electrophoresis might be a reliable approach for the thermally treated shrimp and prawn samples. This study shows that investigating the allergen coding gene might provide a viable way for detecting food-borne allergens in other thermally processed food items, which are becoming more concerned about food safety.

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Introduction

Because of its health-promoting characteristics and ease of processing, seafood has become an important part of the human diet. With a better understanding of the necessity of these crucial animal-based protein sources, global seafood consumption has increased. Shellfish is a significant portion of the seafood sector, with yearly consumption reaching around 4.50 kg per capita globally (Kumar, Yadav et al., 2023). Shrimp and prawns are considered beneficial seafood items that account for approximately 15% of the total market size of the global traded fishery and aquaculture industries, accounting for nearly US$ 43 billion, and this rate is expected to reach approximately 6 million tons per year by 2024 (Khan and Azam, 2021). Similar to other seafood, stir-frying, steaming, boiling, and baking are the most commonly applied processing techniques for both shrimp and prawns. Food-borne allergens can result from consumption, inhalation, or contact with other allergen-specific compounds found in food items (Ward 2015; Nayak; Li et al., 2017). Due to the lack of complete treatment for food allergies, food-borne allergies have been declared the fourth most critical public health problem by the World Health Organization (Amponsah and Nayak, 2018). Seafood allergens, specifically parvalbumin isolated from fish species and tropomyosin isolated from shellfish, have been recognized as major allergens in the food industry and belong in the “big eight” list, which represents the eight food items responsible for approximately 90% of all food-borne allergies. (Nwaru, Hickstein et al., 2014). Shrimp and prawns are responsible for more than 80% of the total occurrences of crustacean allergy, and tropomyosin is accepted as the primary allergenic protein in these species (Ruethers et al., 2018). Due to the thermal resistance characteristic of tropomyosin, several studies have been performed on the determination of the variation in allergenicity driven by different heat processing approaches.

Xu et al. (2020) and Lv et al. (2021) highlighted that stir-frying, boiling, and canning processes lead to variation in the allergenicity of tropomyosin. It's well known that proximate composition, especially fat and protein levels of food items, causes variation in the allergenicity of food items; additionally to protein-based interaction in the allergen mechanism, lipid structures also widely impact the allergenicity of food products (Moreno, 2007). Acidity of food products is another factor playing an important role in the allergen capacity that can differ depending on the properties of the food, processing methods, and storage conditions.

Tiwari, (2004) highlighted the impact of pH on both the allergenicity of almonds and the achievements of allergen detection analyses. Detecting food-borne allergens with a reliable method is the first step in the allergen treatment, elimination, and correct labeling system (Fu et al., 2019). As an alternative to traditional protein-based analyses, DNA-based techniques are mostly preferred due to their high achievement rate caused by DNA being more resistant than protein to heat, pressure, or acidity (Jayasena et al., 2019). DNA-based techniques, especially real-time PCR, have been accepted as official methods by governmental laboratories for the detection of food-borne allergens in Japan and Germany in recent decades (Xu et al., 2020). The achievement of real-time PCR methods in the detection of allergens from various foods, from peanuts to lobsters, has been reported by several researchers; more recently, Aksun Tümerkan (2022) reported that the parvalbumin differences were detected by RT-PCR and confirmed by gel electrophoresis in commercial canned tuna.

Due to the fact that food-borne allergens are considered one of the riskiest fraudulent actions in terms of public health and food security concerns, a better understanding of allergen variations in crustaceans could be useful for both industry and academia. Thus, the aims of this study were to determine the impact of both different thermal processes and proximal differences on the crustacean's allergen. Within these aims, any potential variations in the presence of crustacean allergens depending on thermal processes such as boiling, grilling, frying, baking, and steaming are investigated based on the allergen-encoding gene by RT-PCR and confirmed by gel electrophoresis.

Materials and Methods

Deep-water pink shrimp (*Parapenaeus longirostris, L*) and green tiger prawn (*Penaeus semisulcatus*) were supplied by a local retailer in Izmir, Türkiye, in early 2022. The caught individuals from prawns and shrimp were kept in ice-covered polystyrene boxes and transported to the laboratory directly within the cold chain. Fresh shrimp and prawns were washed in distilled water until they were free of external contaminants. Shells, heads, legs, tails, and veins were removed by a sharp blade prior to further cooking treatments. The total abdominal muscle amount of shrimp and prawns was divided into six groups; one of the sub-groups was stored in raw form and considered the control group.

Thermal treatments

Baking: Edible shrimp and prawn samples are placed on the aluminum foil-covered metal tray and then transferred to the preheated electric oven. The samples were baked at 200°C for 4 minutes (Lasekan and Nayak, 2016).

Frying: 500 g of the shrimp and prawn meats were fried in boiled sunflower oil (1:2, g/mL) for 10 min. in the non-stick deep fryer. The shrimp and prawn samples were stir-fried for 10 min until they became crisp-tender (AlFaris et al., 2021).

Grilling: Shrimp and prawn samples are placed in a gas-operated, aluminum foil-covered oven. The prawn and shrimp samples were grilled individually at 180 °C for 10 min. (Abd-Elghany et al., 2020).

Boiling: 500 g of shrimp and prawn meat and 300 mL of boiling water were poured into a pot for 10 min. in a stainless-steel pan. Then, they were placed on filter paper to drain excess water excess (Abd-Elghany et al., 2020).

Steaming: Edible shrimp and prawn samples are placed on the steamer basket, which is found at the top of the steamer pot. When the water started boiling, the temperature was measured by the probe that was located in the water, reaching up to 100 °C, and then samples were steamed for 10 min. (Khan and Azam, 2021).

After thermal processing, all the moisture and oil were removed by special filter papers. The processed samples were homogenized, stored in sterile falcon tubes, and stored at -80°C until the time of further experiments.

Proximate Composition and pH Measurements

The proximate composition of the raw and thermally processed crustacean samples was analyzed by the following techniques: The crude protein level was calculated according to the Kjeldahl method (AOAC, 1998a), and the conversion factor was accepted as 6.25 for the determination of the crude protein level calculation. The moisture and ash content of raw and processed crustacean samples were analyzed following the AOAC method by the gravimetric method (AOAC, 2000). The total lipid content of the sample was analyzed according to the method of Bligh and Dyer (1959) based on chloroform-methanol solution extraction. The pH of the raw and thermally processed samples was measured using a calibrated 315i/SET pH meter (Weilheim, Germany), as described by Mohan (Mohan et al., 2014).

DNA extraction

DNA was extracted from the raw and cooked shrimp and prawn samples using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with minor modifications such as extending the Proteinase K digestion at 56 ºC. Briefly, a 20 mg grounded sample was lysed with lysis buffer and Proteinase K (Thermo ScientificTM) until no tissue was visible. The DNA pellet was dissolved and applied to the silica column. Then the spin column was washed with AW1 and AW2 solutions. Finally, DNA was eluted with pre-heated elution buffer. The purified DNA was stored at -20 ºC until further experiments.

Quality and yield assessment of Extracted DNA

The DNA yield and quality parameters, such as purity of DNA and the presence of any contaminants in the isolated DNA, were determined by the absorption rate at 230, 260, and 280 nm by a NanoDrop 1000

spectrophotometer (NanoDropTM 2000/2000c, Thermo Scientific, Pittsburgh, PA, USA). All the analyses were performed in triplicate for each group.

Assay design and Real-time PCR

The shrimp tropomyosin gene sequence performed according to the procedure described by Kim et al., (2019), libraries were prepared using as the target sequence a hypervariable 71 bp region by the Clustal Omega program (Table 1). As an internal control, the primer pair was utilized to amplify the 18S rRNA gene region. Real-time PCR was performed in a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). SYBR Green reaction mix and thermal cycles were performed according to the manufacturer's method. The melting curve was acquired by quantifying fluorescence during heating from 65 to 95 °C at a rate of 5 °C per second in the Applied Biosystems system.

Gel Electrophoresis Analysis

Strop-qPCR positivity and the correction of PCR product size were authenticated by gel electrophoresis. 2% agarose gel in Tris-Sodium Acetate-EDTA (TAE) buffer, including 1× SYBR Safe DNA Gel Stain at 100 V. Following the electrophoresis, the gel was visualized in the gel documentation system.

Statistical analysis

The data was analyzed using SPSS 22.0 software (Chicago, IL, USA) by ANOVA and Tukey post-hoc tests at a P value of 0.05 ($P<0.05$) to identify significant variance among the groups. All analyses were performed in triplicate.

Results and Discussion

Proximate composition and pH Differences among Raw and Processed Samples

The proximal composition of crustaceans is changed not only by the properties of the raw material but also by the type of processing methods, which differ by processing time, temperature, and whether there is any external oil or brining solution (Mielcarek et al., 2020). The differences in proximal compositions of raw and thermally processed crustaceans are given in Table 2. The proximal analyses results revealed that the protein level was altered among the raw and processed crustacean samples. A common reduction in protein level can be explained by protein denaturation based on high temperatures during thermal processes (Puthanangadi Dasan et al., 2021). Similarly, moisture and ash levels also decreased during thermal processing in both shrimp and prawn samples, which can be explained by physically removing moisture during the heat process. As seen in Table 2, for the fried samples, the total lipid rate decreased in other processing methods compared to the raw form of the crustacean sample. Statistically significant differences were found in the lipid content between raw and fried shrimp and prawn. This increase can be related to the absorption of external oil during the frying process (Arwani et al., 2022).

Statically important variations were found in the protein, lipid, and ash levels between raw (unprocessed) shrimp and prawn. While the protein content was determined to be 22.1% in raw shrimp, this rate was found to be 24.7% in raw prawn. There were significant differences detected in the thermally processed shrimp and prawn samples, which can be driven by processing temperature and processing time. The highest and lowest crude protein rates were determined in baked (21.4%) and fried samples (5.8%) among the shrimp groups. In the prawn samples, the highest and lowest crude protein rates were found in the steamed (22.6%) and fried samples (6.4%). Relatively higher lipid levels were found in both fried shrimp (16.9%) and fried prawn (12.6%) among the thermally processed samples. The lowest lipid content was found in the grilled shrimp (2.2%) and grilled prawn samples (1.1%). The frying process also significantly decreased ($P<0.05$) the moisture content in both shrimp and prawn samples, which can be related to an increased lipid level and applied heat transfer. Among the thermally processed samples, relatively higher moisture levels were detected in the boiled shrimp (71.4%) and boiled prawn (70.4%). This higher moisture can be related to water retention in the edible part of crustaceans during the boiling process. These results clearly show that the usage of external oil or process temperature directly impacts the proximal composition of crustacean species. The proximal composition has importance for not only nutritional benefits to human health and, consequently, public health, but also for the determination of any hazardous components in the food matrix (Rao et al., 2020). The proximal differences in the raw and processed crustacean samples were detected. Significant differences were found in the raw and prawn samples in terms of protein and lipid levels. Similar differences were also reported in the prawn (*Microtrichium rosenbergii*) and shrimp (*Penaeus monodon) (Islam* et al., 2017). The main differences were observed in the protein and lipid content following the frying process. The protein level decreased and the lipid level increased in both fried shrimp and prawn samples. These differences are in accordance with Arwani et al., (2022) who stated that protein reduction and lipid levels increased following the frying process in the shrimp.

Table 1. Primer sets assessed in this study.

Primer name	Sequence (5' 3') Access no.	Target gene region	Amplicon Base Pair (bp)	Access no.	Reference
StropF	TGTTGGTTGAGCACCTCCTA	Lit v 1 (Shrimp tropomyosin)	71	EU410072	(Kim et al., 2019)
StropR	GCTTCATCGCCTGCATCTTC				
M18S-F	CAGGTCTGTGATGCCCTTAG	18S rRNA	160	-	
M18S-R	GCTTCATCGCCTGCATCTTC				

Table 2. Proximate composition in the raw and processed crustacean samples

Sample	Crude Protein	Total lipid	Moisture	Ash	pH
SR	22.1±0.04^{c}	4.4±0.08^{e}	72.2±1.10^{d}	0.81±0.03^{c}	8.84^{e}
SBA	21.4±0.12^{c}	3.2±0.16^{d}	69.4±1.08^{c}	0.69±0.07^{a}	6.49^{b}
SS	20.9±0.08^{b}	3.0±0.08^{d}	68.2±0.84^{c}	0.67±0.17^{a}	6.72^{b}
SBO	19.6±0.14^{b}	2.6±0.13^{c}	71.4±0.36^{d}	0.75±0.13^{b}	7.05^{c}
SF	5.8±0.20^{a}	16.9±0.07^{g}	59.8±1.20^{a}	0.78±0.09^{c}	7.29^{c}
SG	20.4±0.06^{b}	2.2±0.19^{c}	68.5±1.07^{c}	0.85±0.07^{d}	6.14^{b}
PR	24.7±0.14^{d}	2.4±0.12^{c}	71.5±0.07^{d}	0,89±0.21^{e}	8.50^{d}
PBA	21.4±0.16^{b}	2.2±0.08^{c}	63.9±0.27^{b}	0.81±0.07^{c}	6.58^{b}
PS	22.6±0.22^{c}	1.8±0.04^{b}	65.7±0.16^{b}	0.71±0.03^{b}	6.29^{a}
PBO	20.3±0.07^{b}	1.4±0.08^{b}	70.4±0.16^{d}	0.84±0.08^{d}	7.16^{c}
PF	6.4±0.12^{a}	12.6±0.18^{f}	58.6±0.22^{a}	0.81±0.11^{c}	7.24^{c}
PG	21.7±0.18^{b}	1.1±0.05^{a}	64.8±1.28^{b}	0.76±0.12^{b}	6.05^{a}

SR: Raw shrimp, SBO: boiled shrimp, SF:fried shrimp,SBA:baked shrimp,SS steamed shrimp,SG:grilled shrimp,PR:raw prawn, PBO: boiled prawn, PF: fried prawn, PBA:baked prawn,PS:steamed prawn,PG:grilled prawn.Data are expressed as mean value ± standard deviation of triplicates. Values followed by different letters indicate significant differences (P<0.05) Values in a same column followed by different numbers indicate significant differences of the parameter with respect to groups

Table 3. DNA yield and Quality of raw and processed forms of crustacean samples

Sample	DNA Yield (µg/µL)	Purity (A260/A280)	Chemical Contamination (A260/A230)
SR	1551.20±0.13^{e}	2.02±0.02^{c}	2.30±0.01^{c}
SBO	556.70±0.02^{c}	2.10±0.04^{c}	2.30±0.02^{c}
SF	1189.10±0.06^{e}	2.06±0.02^{c}	2.30±0.05^{c}
SBA	842.70±0.08^{d}	2.10±0.03^{c}	2.11±0.04^{c}
SS	562.40±0.04^{c}	2.00±0.01^{c}	2.28±0.02^{c}
SG	1458.10±0.05^{e}	2.10±0.02^{c}	2.30±0.05^{c}
PR	52.50±0.04^{b}	1.26±0.03^{b}	-0.59±0.02^{b}
PBO	4.60±0.03^{a}	1.10±0.04^{b}	-0.70±0.02^{b}
PF	12.50±0.04^{a}	1.46±0.02^{b}	20.86±0.04^{d}
PBA	5.40±0.04^{a}	2.00±0.01^{c}	4.03±0.03^{c}
PS	1.00±0.04^{a}	0.62±0.02^{a}	-1.98±0.02^{a}
PG	4.00±0.04^{a}	1.15±0.03^{b}	-0.59±0.07^{b}
PR	52.50±0.05^{b}	2.02±0.04^{c}	2.30±0.03^{c}

SR: Raw shrimp, SBO: boiled shrimp, SF:fried shrimp,SBA:baked shrimp,SS steamed shrimp,SG:grilled shrimp,PR:raw prawn, PBO: boiled prawn, PF: fried prawn, PBA:baked prawn,PS:steamed prawn,PG:grilled prawn.Data are expressed as mean value ± standard deviation of triplicates. Values followed by different letters indicate significant differences (P<0.05) Values in a same column followed by different numbers indicate significant differences of the parameter with respect to groups

The pH differences in the raw and thermally processed shrimp and prawn samples are presented in Table 2. The higher pH values were determined in the raw form in both shrimp and prawn samples (8.84 and 8.5, respectively). The pH values of processed samples decreased among all thermally processed groups, regardless of the crustacean species. The highest pH values were determined in the fried shrimp (7.29) and fried prawns (7.24) among the thermally processed groups. This relatively higher pH value can be correlated with the lowest moisture level and denatured protein rate (Rabie et al., 2016). The sharp decreases were observed in the grilled sample, with 6.14 and 6.05 for grilled shrimp and grilled prawn, respectively. Relatively higher pH value and lowest protein level fried sample in both shrimp and prawn revealed that the pH impact on the protein level of the food sample Immersion of vapor, heat penetration, and interaction of other compounds cause variations in the protein structures and, consequently, the allergenicity of food (Chi and Cho, 2016). Venugopal and Gopakumar (2017) highlighted that boiling and steaming processes also affect the proximate composition of crustaceans. The pH level also changed depending on the thermal processing methods. The pH level decreased following all processing methods in both shrimp and prawn. This reduction is in accordance with Bello (2013), who reported that the pH level decreased from 8.50 to 4.50 depending on heat treatment in the prawn.

The yield and quality of extracted DNA

While the DNA yield has no effect on the PCR amplification, it is one of the key factors impacted by the thermal processing of food samples. The DNA yield was determined with the sample weight, DNA concentration, and the final volume for each group. The DNA yield of raw forms was determined to be significantly higher than that of thermally processed shrimp and prawn samples. Surprisingly, higher DNA yields were observed in all shrimp groups, regardless of whether they were raw or thermally processed, than in prawn groups; these significant differences revealed that even the same pre-treatments or extraction methods were applied to samples. The yield of extracted DNA can vary from species to species, which could be related to the thermal integrity of the raw material. In the processed shrimp and prawn sample groups, DNA yields differed depending on thermal treatment. These significant differences can be explained by the processing time and temperature that limit the DNA extraction process. The highest DNA yield was determined from the steamed samples (1458.10 µg/µL) and the prawn samples (12.90 µg/µL) for shrimp and prawn samples (Table 3). The relatively lower DNA yield determined from the grilled sample in both shrimp (502.40 µg/µL) and prawn samples (1.02 µg/µL) These significant variances can be explained by DNA degradation.

The purity of DNA from raw and thermally processed shrimp and prawn samples is shown in Table 3. The purity of shrimp samples was found to be relatively lower in the prawn samples that were processed with the same procedure, even in the raw form of the crustacean samples. The purity of DNA from raw and thermally treated prawn groups was found to be statistically different, and the highest and lowest purity values were found in the baked and steamed prawn samples, respectively. These differences can be explained by the different levels of thermally degraded DNA (Quintrel et al., 2021). Another key factor in the achievements of DNA-based methods in the food industry is the A260/A230 ratio, which indicates the presence of contaminants such as salts, oils, or carbohydrates (Babić et al., 2018). Among all the raw and thermally processed shrimp and prawn groups, the highest contaminants rate was found in the fried shrimp (22.30) and fried prawn (20.86), which can be related to the use of external oil during the frying process (Table 3). The optimal limits (2.0–2.2) were not detected in any of the prawn samples. These significant variances revealed that the same processing techniques can result in differences in DNA quality even in cryptic species. Thermal processing heavily affects the quality and integrity of isolated DNA from food items, especially seafood, which is known to be highly perishable due to quality degradation (Tsai et al., 2023). The quality loss of extracted DNA can directly result from the heat treatment, and the presence of any additives during processing can also increase the intensity of DNA fragmentation, which may cause a lack of amplification (Pascoal et al., 2008). The lowest DNA yield was found in the grilled samples, regardless of the crustacean species. These results agree with Tümerkan (2021), who reported that the lowest DNA yield was found in the thermally processed fish sample. The purity of DNA from raw and processed groups was found to be statistically different. These differences are consistent with the result of Musto (2011), who proved that the variances in the DNA quality and integrity of animal-based protein sources are impacted by various cooking methods. The highest contamination rates were found in the fried samples, which could be attributed to the damaging effects of oil accelerated by heat treatment on the raw samples during the frying process. This result is in agreement with previous findings that the frying process impacted the detection limit of food items in DNA-based methods (Eischeid, 2019). These findings were generally in agreement with other research interpreting the differences in the allergenicity of crustacean and mollusk species driven by thermal processing approaches.

Tropomyosin Gene Detection as a Marker for Crustacean Allergens in Raw and Processed sample

In this research, crustacean allergen-coding sequences were used as targets for reliable molecular methods for the detection of shrimp and prawn allergens in both raw and thermally processed samples. For that purpose, five different cooking methods were applied to both shrimp and prawn samples. Due to the less than 100-bp gene region, it commonly reduces the DNA degradation in highly processed food (Lit V3) gene regions used for tropomyosin detection (Rao et al., 2020). To confirm, experimentally, an internal primer (M18S) 18S rRNA was used for testing the specificity of the primers. Fig. 1 presents the assay of PCR fluorescence plots, including melting curves, and the amplification for both tropomyosin (Fig. 1.A, C) and internal control (Fig. 1.B, D), respectively. These results clearly demonstrated that, allergen-coding gene region can be used alternatively for determination of the presence of the food-borne allergen in the dfferent matrices.

Any tropomyosin-encoding gene was not detected in the raw form for shrimp and prawn; sharp peaks with identical melting points were observed by melting curve analysis in the fried and grilled shrimp and prawn samples. As seen in Figure 1.A, relatively higher Tm values were found in the grilled and fried shrimp and prawn samples (G9, G10, G11, and G12).

Fried and grilled samples were determined to be allergen-positive groups, which means the different processing techniques impact the allergenicity of crustacean samples. As seen in Fig. 1.C, clear distinctions between amplicons based on tropomyosin-encoding genes were determined. The results of the melting curve and amplicons clearly demonstrate that allergen-encoding genes were detected by the RT-PCR technique. Owing to the achievements of RT-PCR in the allergen coding gene, a wide range of research has been conducted to determine foodborne allergens in different products without any protein-based method confirmation. For example, Sanchiz et al., (2021) and Torricelli et al., (2020) reported reported the allergen encoding gene in the various food samples, such as peanut, sesame, and pistachio, was changed by different processing methods. More recently, Aksun Tümerkan (2022) highlighted the achievements of fish allergen encoding gene detection by RT-PCR. Due to gel electrophoresis, confirmation plays an important role in the achievements of RT-PCR analyses to prevent any misinterpretation resulted by SYBER-green dye. Following the RT-PCR, the allergen-encoding gene was also analyzed on the gel electrophoresis to confirm. As seen in Figure 2, clear bands were detected in the fried and grilled shrimp and prawn samples, which obviously confirmed the specificity of the RT-PCR and agarose electrophoresis techniques. There was no band observed in the other raw and thermally processed samples.

The results of gel electrophoresis are in accordance with Li et al., (2021) who reported the achievements of allergens in both dairy products and shrimp balls using the same techniques. Interestingly, (Khan et al., 2019), highlighted that the thermally degraded tropomyosin can be recovered by cooling at the ambient temperature (~25°C). This important characteristic of tropomyosin has increased attention for food safety due to the fact that shrimp and prawns can be consumed at room temperature. Structural changes of allergen-response proteins occur influenced by the properties of the food matrix, applied processing methods, and storage conditions until they reach consumers.The alteration of allergens driven by thermal treatment in food products is commonly caused by the peptide bonds hydrolysis, aggregation of both disulfide and non-covalent bonds and denaturation of allergen respond mechanisms (Laly et al., 2019). The tropomyosin coding gene were found in the fried and grilled crustacean samples. While, Shriver et al., (2011) claimed that boiling of shrimp did not change the tropomyosin, other researchers reported that major heat-resistance allergen was found in the boiled, giant river prawn and shrimp samples(Lasekan and Nayak, 2016).

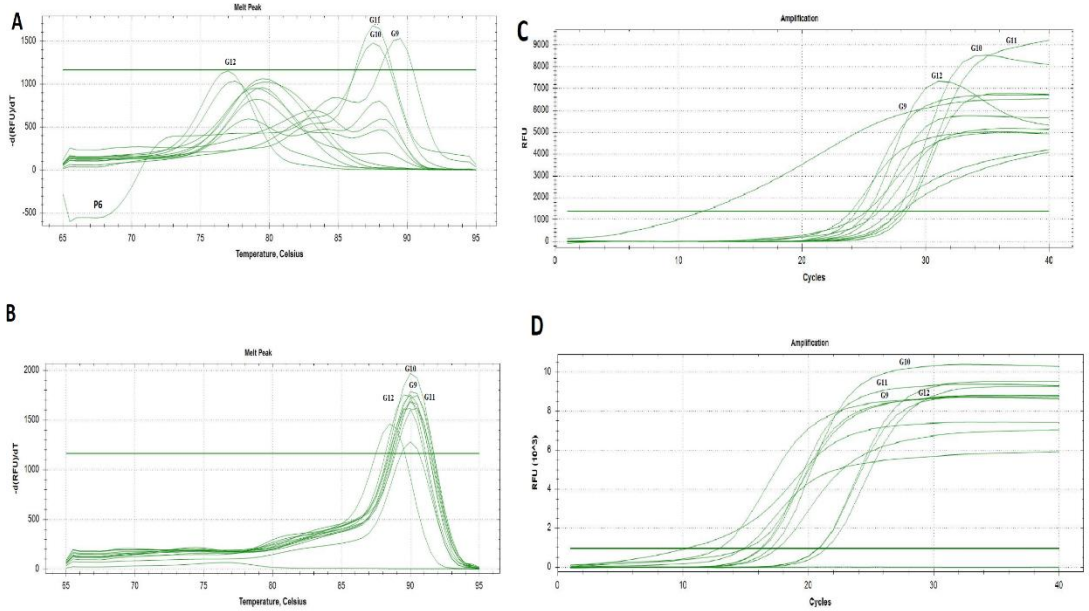

Figure1. Melting curves (A, B) with Sybr-Green dye targeting the Litv3 and M18s of crustacean sample, Real-time PCR amplification (C, D)

(G1: Raw shrimp; G2: raw prawn; G3: Baked shrimp; G4:Baked prawn; G5:Steamed shrimp; G6:Steamned prawn; G7:Boiled shrimp; G8:Boiled prawn; G9:Fried shrimp; G10: fried prawn; G11:Grilled shrimp: G12:Grilled prawn)

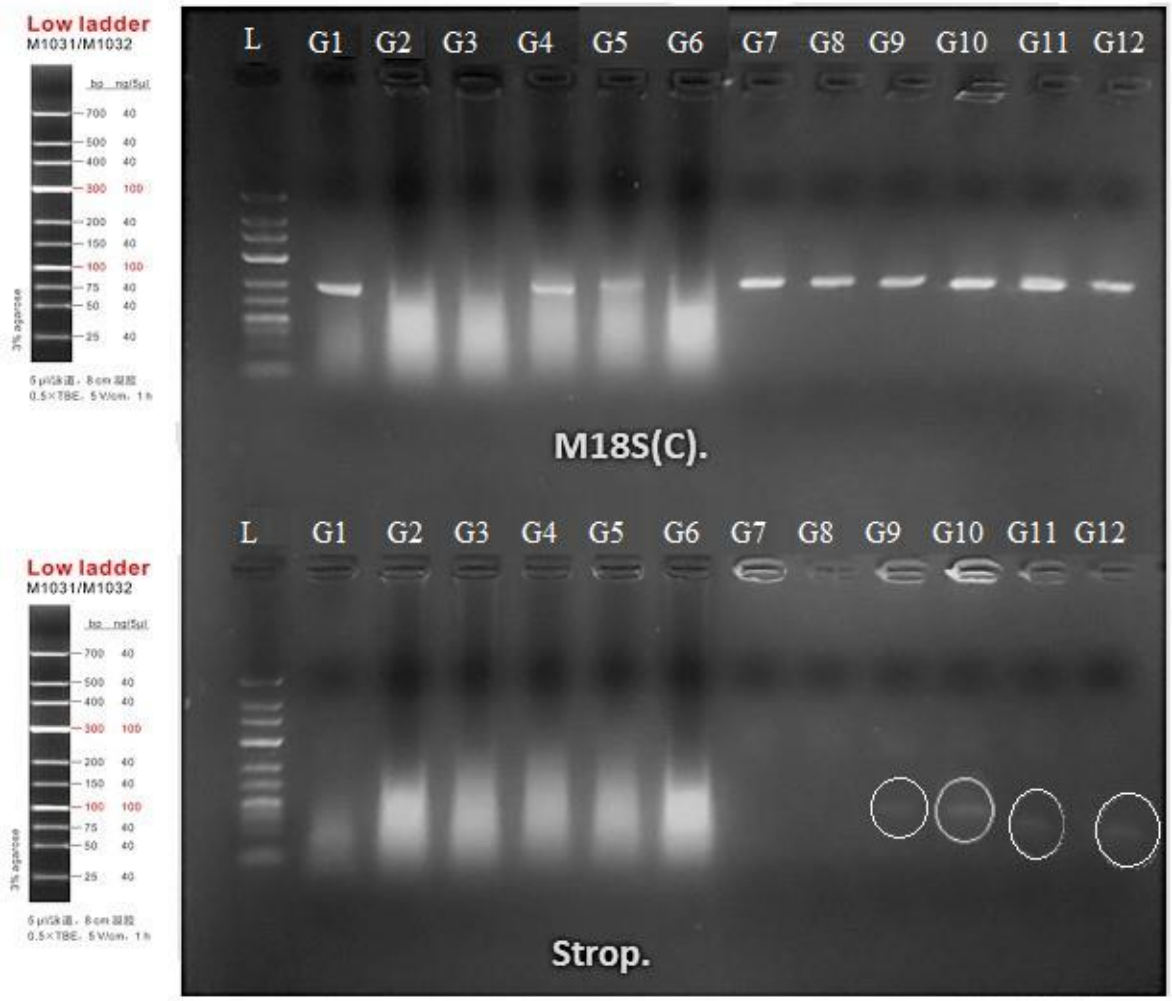

Figure 2. Gel electrophoresis of amplified tropomyosin and internal standard from raw and thermally processed samples

(G1: Raw shrimp; G2: raw prawn; G3: Baked shrimp; G4:Baked prawn; G5:Steamed shrimp; G6:Steamned prawn; G7:Boiled shrimp; G8:Boiled prawn; G9:Fried shrimp; G10: fried prawn; G11:Grilled shrimp: G12:Grilled prawn)

Likewise, the higher stability of other main crustacean allergens, myosin light chain (Lit v 3) reported in the boiled sample (Ayuso et al., 2008).

More recently, Laly et al., (2019) reported that the identification of al-allergenic properties of tropomyosin during extended boiling was identified in flower tail shrimp. The results of this research also revealed the presence of tropomyosin encoding gene were found in fried and grilled crustacean sample. This result is in accordance with Lasekan(Lasekan and Nayak, 2016),who reported that grilling process increased tropomyosin level of shrimp (*Penaeus monodon*). These results are important for better understanding of how thermal process impact on the proximate composition and acidity of seafood that play important role in the DNA based allergen detection. The results could be valuable for the determination of the other food consumption originated allergens which are accepted as one of the important threats for the food safety and thereof public health. The alteration of allergens driven by thermal treatment in food products is commonly caused by peptide bond hydrolysis, aggregation of both disulfide and non-covalent bonds, and denaturation of allergen response mechanisms (Laly et al., 2019). The tropomyosin-coding gene was found in the fried and grilled crustacean samples. While Shriver et al., (2011) claimed that boiling shrimp did not change tropomyosin, other researchers reported that major heat-resistance allergens were found in the boiled giant river prawn and shrimp samples (Lasekan and Nayak, 2016). Likewise, the higher stability of other main crustacean allergens, myosin light chain (Lit v. 3), was reported in the boiled sample (Ayuso et al., 2008). More recently, Laly et al., (2019) reported that the identification of al-allergenic properties of tropomyosin during extended boiling was identified in flower tail shrimp. The results of this research also revealed the presence of tropomyosin-encoding genes in fried and grilled crustacean samples. This result is in accordance with Lasekan and Nayak, (2016), who reported that the grilling process increased the tropomyosin level of shrimp (*Penaeus monodon*). Taki et al., (2023) highlighted that the imposrtance of molecular based methods on the detection of allergen in the different food matrices. Jabeen et al.,(2023) also pointed out that allergen detection in cereals by real-time PCR. The results of this research are important for a better understanding of how thermal processes impact the proximate composition and acidity of seafood, which play an important role in DNA-based allergen detection. The results could be valuable for determining the other food consumption-derived allergens, which are accepted as important threats to food safety and public health.

Conclusions

The findings of this research highlight that the tropomyosin encoding gene can be used for the crustacean allergens using different thermal processing methods, even in cryptic or close species. The proximate composition and pH values of raw and processed shrimp and prawn samples were also found to be significantly different. The allergen-coding gene was found in fried shrimp and prawn samples that could be related to the external oil effect. Grilled shrimp and prawn samples were other tropomyosin-positive groups, which could be related to pH variations. This study is the first to achieve the simultaneous analysis of allergenic crustacean species driven by thermal process effects with molecular methods in different crustacean species in the same research. The findings relevead that different thermal processing impact both on the nutritional value and allergenity which are crucial for public health.Due to the thermal processing methods applied by both consumers and producers, the outpots of this research could be beneficiary in domestic and industry level. The results could be beneficial for food processors, scientists, and decision-makers in public health. The scope of further research will be the stability of foodborne allergen detection and the allergen-detectable signal contained in packaging material.

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Energy Efficiency Analysis in the Case of Sustainable Lighting Design Butterfly Valley Park (Konya)

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ABSTRACT

The concept of lighting is of great importance in providing the security that people need so that they can have a quality of life and in meeting their aesthetic needs. Lighting design is related to the physical structure and the environment, and thanks to the lighting design, the aesthetic and functional needs of people will be met and the quality of life will be increased. The need to reflect the historical, cultural and aesthetic values of the city has brought the issue of urban lighting to the agenda. Lighting systems, which are applied without paying attention to the necessary lighting standards and criteria, cause some problems in urban spaces. One of these problems is light pollution; It is generally defined as the use of light in the wrong place, in the wrong amount, in the wrong direction and at the wrong time. Light pollution adversely affects the natural life and daily lives of people, and it is necessary to take various measures to eliminate such negativities in human life, to detect and eliminate these problems. In this study; The standards for the lighting of the city parks obtained by the literature studies were determined, the current lighting situation of the Butterfly Valley Park and the recommended lighting design produced by the Relux software in accordance with the standards. According to the findings; In the current lighting project, the total power consumed as a result of the lighting of all the lamps of the area has been calculated as 96 005.0 W. In the proposed lighting project, the total power consumed as a result of the burning of all lamps was calculated as 27 630.0 W. The obtained energy gain was found to be 96 005.0 W – 36 700.0 W = 68 375.0 W. As a result of working in the light of this information, energy savings of 1 in 3 have been achieved.

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Introduction

Light is a fundamental source of energy, and in today's world, it is inconceivable to imagine life without it (Aksay et al., 2007). The 20th century witnessed a significant increase in urbanization and technological advancements, leading to new developments in the field of lighting. Lighting is essential not only for illuminating urban areas but also for purposes such as road lighting, billboard illumination, and security.

Furthermore, the need to showcase the historical, cultural, and aesthetic aspects of a city has brought urban lighting into the spotlight. Urban lighting not only serves functional purposes but also emphasizes the distinctive qualities of the city's architecture and ambiance (Dokuzcan, 2006). As a result, lighting applications should be designed to meet specific needs and serve their intended purposes effectively.

It is crucial to emphasize that a successful lighting design should take into account human psychology and physiology (Alişan, 2013).

According to Ataç (2008), one of the fundamental requirements for ensuring the well-being of individuals is the provision of a safe living environment. This necessitates not only the meticulous planning of physical spaces but also the deliberate inclusion of safety measures within urban planning. Inadequate lighting during the late hours of the night can transform urban areas into high-risk zones for criminal activities. Therefore, it becomes imperative to formulate urban spaces that prioritize safety as a means to mitigate crime and the associated fear that permeates the citizenry, as highlighted by Yenioğlu (2010).

In this context, lighting emerges as one of the most effective and appropriate reinforcing elements capable of fulfilling this crucial role in urban safety enhancement.

Incorrect or insufficient lighting installations not only fall short of establishing an aesthetically pleasing atmosphere but also lead to wasteful consumption of energy, as noted by Demircioğlu Yıldız and Yılmaz (2005). An effective lighting design seeks to provide the exact

amount of illumination needed for a particular space (Güngör et al., 2019). It is evident and unequivocal that illuminating unoccupied areas or surpassing the necessary lighting levels in occupied spaces results in significant and unnecessary energy wastage, as emphasized by Rajkhowa (2014), Meier et al. (2014) and Güngör and Öner (2020).

In the present era, urban street lighting alone accounts for approximately 4% to 6% of a city's total energy consumption. However, when considering the energy expended on illuminating building facades, advertisements, and billboards, this percentage can escalate to as much as 10%. Research conducted by the International Dark Sky Association reveals that a concerning 30% of the energy consumed in these lighting practices is wasted (Çetin et al., 2003). This proliferation of misguided lighting approaches, flawed lighting system designs, and the underutilization of advancing lighting technologies has given rise to light pollution, which is now recognized as a significant form of pollution in the contemporary world (Önder and Konaklı, 2002).

In the fields of Landscape Architecture and Architecture, a noticeable deficiency exists in adequately recognizing the significance of light. Neglecting the pivotal role of light, which is an essential element in foundational design principles, across various applications can result in shortcomings in Landscape Architecture endeavors, as emphasized by Demircioğlu Yıldız and Yılmaz (2005).

It's worth noting that one of the crucial elements in landscape architecture is the inclusion of plants. The absence of adequate light or insufficient illumination can have detrimental effects on the vital life activities of plants, causing them to undergo stress. Therefore, light serves as the source of life not only for humans but also for plants and, by extension, for all living organisms, as articulated by Çorbacı et al. (2012).

Light pollution, a prominent urban environmental predicament, stands as a substantial ecological challenge that undermines landscaping initiatives. Light serves as a tool facilitating enhanced visual perception of our surroundings and cultivating a heightened sense of security among urban inhabitants. In succinct terms, the concept of "light pollution" pertains to the inappropriate deployment of light in terms of location, quantity, and form. The misuse of light encompasses superfluous energy wastage stemming from erroneous lighting designs (Kellert, 2008).

The primary objective of this study is to propose viable solutions addressing the challenges identified through a comprehensive analysis of outdoor lighting and light pollution, informed by a thorough examination of pertinent literature. The underpinning theoretical framework has been established by delving into subjects encompassing outdoor lighting's conceptual foundations, its established standards and methodologies, challenges encountered during both the design phase and practical implementation, along with the corresponding remedial measures. This exploration extends to artificial light sources, comparative evaluations of lighting sources, illumination master planning, and lighting techniques specific to urban parks.

The study endeavors to showcase the contemporary lighting project undertaken at the Butterfly Valley Park, situated within the confines of the Selçuklu District of Konya Province, and subsequently contrast it with a proposed park lighting project meticulously aligned with established standards. As part of the research scope, the areas within the park afflicted by inadequate lighting and light pollution were identified. Furthermore, the quantification of energy loss stemming from light pollution was computed using specialized software applications.

Material and Method

The main material of the research is Butterfly Valley Park located in Selçuklu District of Konya Province. Butterfly Valley Park (latitude and longitude) is located at 37° 56' 54.61", 32° 27' 42.45" coordinates. Located within the borders of Sille Parsana District, the park has an area of 385 thousand square meters. The location of Butterfly Valley Park is shown in Figure 1 with satellite images taken from Google Earth Pro and Konya City Guide application.

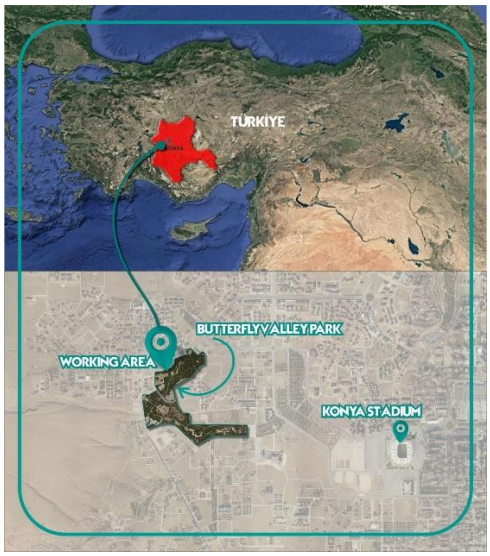

Figure 1. Google Earth Location of Konya and Butterfly Valley Park

To create the theoretical foundations;

The research material for this study comprises various sources and tools. These include the existing literature on the subject, structural and landscaping project documents obtained from the Butterfly Valley Park administered by the local government, lighting design projects, detailed plans, quantity survey files, and related materials. Additionally, data collected during face-to-face interviews, satellite imagery of the research area captured during both daytime and nighttime, photographs, catalogs, and information obtained from lighting element companies and online sources will be utilized.

In terms of software applications, the research will employ Autocad, Relux, Microsoft Office, Photoshop, Corel Draw, and, when necessary, SketchUp, Lumion, and 3D Studio Max computer programs to analyze, visualize, and present the research findings.

The utilization of lighting design software programs has significantly increased in recent times, with many programs now offering practical luminance measurement capabilities for the luminaires entered into the software, as noted by Erten (2014). To achieve higher levels of energy efficiency and savings, it is imperative to calculate

potential gains accurately, especially during the preliminary phases of indoor and outdoor lighting design, as emphasized by Uygun and Görgülü (2016).

Compared to traditional mathematical methods, lighting calculations performed using computer programs result in fewer errors and yield more accurate outcomes, as mentioned by Buyukbicakci (2010). Furthermore, developing the lighting design of a project within a computer environment and conducting measurements using these tools not only saves time but also proves to be cost-effective (Dursun, 2005).

In this context, the Relux program has been chosen as the primary tool for this study due to its widespread use in recent years for lighting design and calculations. The Relux program allows the exchange of files with CAD-based programs in Windows metafile, dxf, 3ds, and ASCII formats. Additionally, ReluxCAD 2.0, which operates in AutoCAD versions from 2000 to 2022, generates drawings within the AutoCAD program and facilitates the transition to the Relux program for calculations. It's worth noting that the existing Reluxcad 2.0 is also compatible with AutoCAD 2022 (Işık, 2009).

To gather the necessary information for the study area, a comprehensive approach was employed. This involved examining multiple sources, including articles, projects, theses, papers, reports, maps, and other relevant documents, both from local and international contexts, related to fields akin to the subject of the study. The review of literature and documents encompassed several aspects, including the concept of lighting, the utilization of lighting in outdoor and open-green spaces, and general design standards. Furthermore, the research involved the examination of design and application examples derived from similar studies.

The initial phase of the research began with the delineation of the research area, a process that entailed clarifying the study's objectives and scope. The establishment of theoretical foundations was achieved through extensive reviews of both domestic and foreign literature.

The second stage of the research involved the creation of a current lighting project using the Relux software. This was accomplished by utilizing the existing lighting project of the research area to identify areas susceptible to light pollution and lighting deficiency. The identification of regions afflicted by light pollution and insufficiency was accomplished by superimposing the lighting maps obtained from the software.

Moving on to the third stage of the research, an energy efficiency assessment was conducted using the Relux software. Utilizing the consumption data acquired, comparisons were made between the current lighting situation and the proposed plan in accordance with established standards. Any potential energy losses during usage periods were quantified and assessed.

Finally, in the last stage of the research, an in-depth analysis of the gathered data was undertaken. Following the analysis, discussions, conclusions, and recommendations were formulated based on the outcomes of the study.

Consequently, the illumination levels for Konya Butterfly Valley Park were determined based on the existing lighting plan. The initial project parameters (lm/m²-lux) from the Autocad program were transferred to the Relux software for further calculations. The current lighting map for Konya Butterfly Valley Park was generated using the illuminance data obtained through the lighting software.

The positions of the lighting fixtures specified in the lighting project provided by the local government were integrated into the Relux program, taking into consideration the appropriate models, luminaires, and power specifications. Subsequently, all the luminaires within the software were activated. Ultimately, the current lighting map was formulated utilizing the calculation tools within the software. Throughout this process, the characteristics and brands of the luminaires were duly taken into account.

The luminance values, which were entered into the software based on the existing luminaire types, sizes, brands, and their respective locations within the park, were used to construct a luminance map. Subsequently, with the aid of the Relux software, all luminaires within the current lighting map were operated to generate an illuminance level map.

Photographs were taken both during the day and at night at various times within the park to ascertain the locations and types of luminaires utilized in the study area. The lighting elements in Butterfly Valley Park were evaluated with regard to their functional, aesthetic, and technical attributes, as well as their brightness levels and energy efficiency. These assessments were conducted in accordance with established standards for various park areas, including pedestrian and bicycle paths, recreational zones, children's playgrounds, sports facilities, flower gardens, seating and gathering areas, entrances, and parking lots.

Following the evaluation of the current lighting map and the proposed lighting map for Butterfly Valley Park, an analysis was conducted to determine the energy savings achieved by the park. Additionally, the daily and monthly electrical energy consumption was calculated and expressed in terms of the equivalent traditional TL (Turkish Lira) currency.

Results and Discussion

Established in 2015, Butterfly Valley Park is situated within the Selçuklu District of Konya Province. It is located within the confines of the Sille Parsana District and spans an expansive area of 38.5 hectares. The park boasts a notable attraction known as the Konya Tropical Butterfly Garden, which consists of four distinct components. These components include a production zone where each stage of the butterflies' life cycle, from pupa to expiration, is observed, an enclosed flight area, an open flight zone, and a butterfly museum. The park encompasses a vast expanse of 270 000 m² of green space, complemented by 105 thousand square meters of paved surfaces, and an additional 10 000 m² dedicated to aquatic features.

Furthermore, Butterfly Valley Park offers a diverse range of amenities, including an amphitheater, a miniature cinema, ponds, and water features, exercise equipment installations, children's play areas, circus, and go-kart sections, as well as a greenhouse. Figure 2 provides an illustration depicting the structural implementation plan of the designated research area, Butterfly Valley Park.

Figure 2. Butterfly Valley Park Structural Application Project

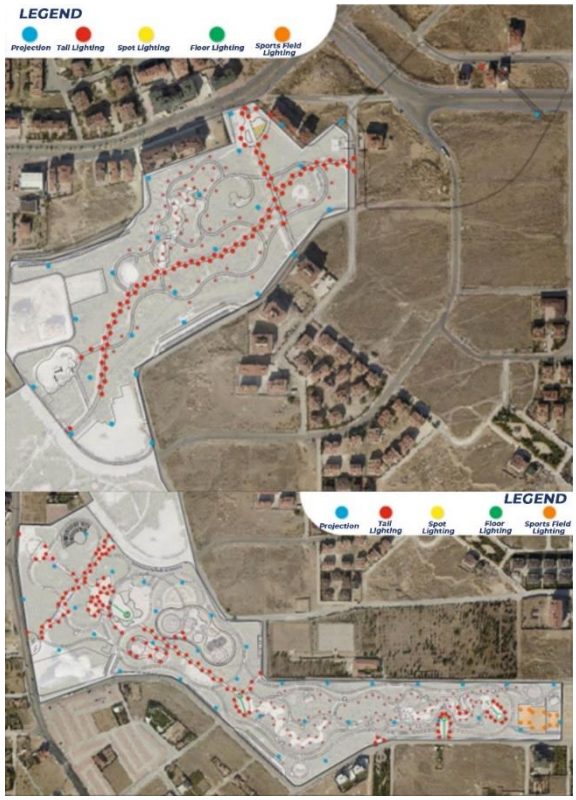

Figure 3. Locations of Existing Lighting Elements in Butterfly Valley Park

Butterfly Valley Park, situated within the open-green area framework of the Selçuklu district in Konya province, is conceived as a recreational destination catering to diverse activities and prioritizing elements such as accessibility. It was meticulously designed as a haven for repose and amusement, aligning with fundamental principles of park design.

In the landscape architecture design of Butterfly Valley Park, the layout was developed based on input and guidance from local authorities. As a result, the park's composition includes a total of 14 433 coniferous trees belonging to 7 distinct species, 10 956 broad-leaved trees representing 21 different species, 18 810 evergreen shrubs and bushes sourced from 23 diverse species, 5 699 foliage elements representing 19 various species of verdant shrubbery, and 50 875 ground cover flowers encompassing 6 discrete species. The park, with its vast green expanse covering 270 000 m², also features a vibrant flower garden adorned with a multitude of colorful botanical specimens.

Lighting Project and Current Situation of the Park

The lighting scheme for Butterfly Valley Park was conceptualized and executed by the Selçuklu Municipality. Subsequently, comprehensive assessments were conducted based on the received projects. The existing lighting fixtures within Butterfly Valley Park were visually documented, mapped, and categorized based on the observations made within the study area. This process involved on-site examinations, consultations with authorized individuals, and an examination of the provided lighting project.

The inventory of available lighting elements and their colors on the map are as follows:

- Tall Lighting – 208 poles length 4.5 m and power MHR-150W Bulb luminaire (Red)
- Projector – 62 pieces of mast length 20 m and power MHR-250W x 4 pieces of Bulb luminaire (Blue)
- Spot Lighting – 24 underground 27 cm above ground 23 cm and 5W luminaires (Yellow)
- Floor Lighting – 71 x 5W Power LED luminaires (Green)
- Field Lighting - 20 poles 6-12 m in length and 250Warmatur (Orange)

The distribution of the above luminaires in the Butterfly Valley Park;

- 208 number 1 lighting elements around the walkway,
- 62 number 2 lighting elements distributed homogeneously throughout the area,
- 24 lighting elements numbered 3, located under the trees in two different areas,
- 71 number 4 lighting elements used in squares,
- 20 number 5 lighting elements positioned on the poles inside the sports fields,

In Figure 3, the location of the lighting elements in the Butterfly Valley Park is shown with their colors.

Analysis of the Current Lighting Condition of the Park

While Butterfly Valley Park is a relatively recent addition, it has come to our attention that certain areas within the park suffer from inadequate lighting due to malfunctioning existing lighting fixtures. Conversely, areas where maintenance and repair have been carried out exhibit improved lighting conditions. Notably, the newly installed lighting elements showcase a modern aesthetic that seamlessly integrates with the park's design. These elements have been carefully sized and positioned in relation to pedestrian walkways, cycling paths, footpaths, green spaces, and trees, ensuring a harmonious blend with the park's natural environment.

However, the presence of high mast projectors scattered throughout the park has introduced an aesthetic imbalance, hindering the creation of a visually pleasing

ambiance within the park. Concerning their installation, the lighting fixtures in Butterfly Valley Park are anchored to concrete bases, but a discernible pattern in their arrangement is lacking. Upon activating the existing lighting fixtures in the Relux program, the resulting visual representation corresponds to the depictions presented in Figure 4 and Figure 5.

Proposal Lighting Project for Butterfly Valley Park

The results generated by activating the lamps positioned throughout the design layout and analyzed using the Relux software are illustrated in Figure 6. With all the lamps illuminated, the cumulative luminous flux emitted by the lamps amounts to 5,167,812.00 lumens, and the total power consumption measures 27,630.0 watts (W).

The inventory of the proposed lighting elements and their colors on the map are as follows:

1. Tall Lighting – 261 poles length 4.5 m and power MHR-30W Bulb luminaire (Red)

2. Projector – 10 pieces of mast height 20 m and power MHR-250W x 4 pieces of Bulb luminaire (Blue)

5. Field Lighting - 12 poles length 6-12 m and 200 W luminaire (Orange)

General

Calculation algorithm used	: Average indirect part
Maintenance factor	: 0.80
Total luminous flux of all lamps	: 5167812.00 lm
Total power	: 27.630.0 W
Total power in the area (1064972.00 m²)	: 0.03 W/m²/ (100lx)

Energy Gains Obtained from Butterfly Valley Park with Proposal Lighting Project

With the preparation of current and proposed lighting maps of Konya Butterfly Valley Park, the electrical energy consumed in the park was calculated. When the amount of power consumed as a result of the current and recommendation map prepared in the Relux program is compared, it has been revealed that the total power obtained with the recommendation map is approximately 1/3 more efficient than the current one (Table 1). The proposal lighting project, prepared in accordance with the standards, reveals the importance of the study as it brings energy efficiency to the fore and saves energy, which is one of the current problems of the study.

In the current lighting project, the total power consumed as a result of the lighting of all the lamps of the area has been calculated as 96005.0 W. In the proposed lighting project, the total power consumed as a result of the burning of all lamps was calculated as 27630.0 W. The obtained energy gain was found to be 96005.0 W – 36700.0 W = 68375.0 W.

As a result of the power consumption over a 1-hour period in the project, the energy consumed in 1 hour amounts to 27 630 watts (W). The calculation conducted for the month of August, which is typically when parks are most frequently used, yields a total energy consumption of 273 500 W between the evening hours (21:00-01:00), multiplied by 4, to be 1 093 000 watts or 1 093 kW for 1 day. Consequently, the electrical energy consumed over 1 month is calculated as 1 093 kW multiplied by 31 days, which equals 33 883 kW or 33 883 000 watts.

Figure 4. Night Intensity Map of Existing Illuminations

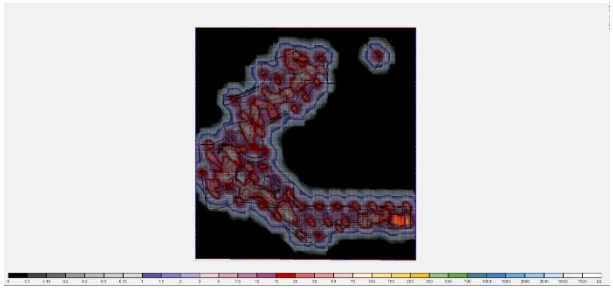

Figure 5. Illuminance Level Map of Existing Illuminations

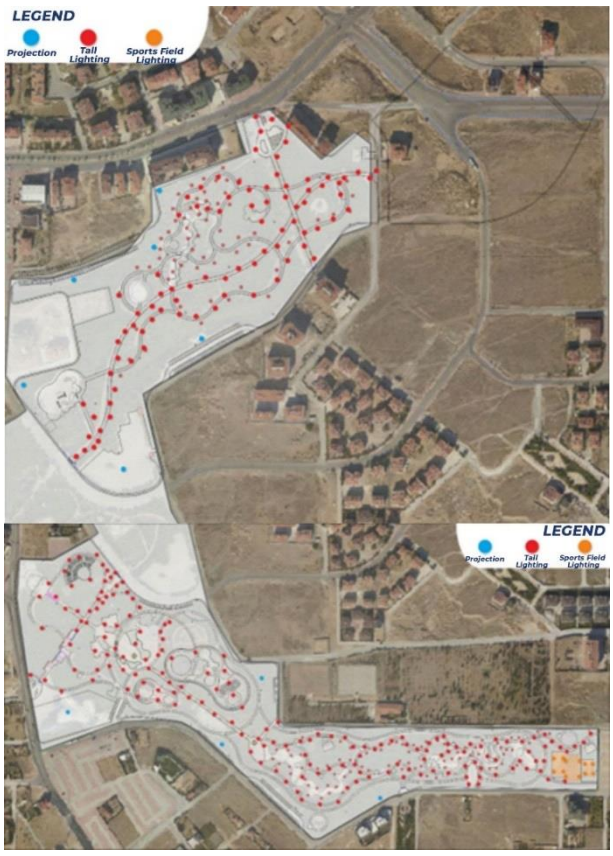

Figure 6. Locations of Butterfly Valley Park Proposed Lighting Elements

In accordance with the market prices obtained from MEDAŞ (Meram Electricity Retail Sales Joint Stock Company), it is evident that for 1 watt of electrical energy used within the park, up to 240 kWh costs 1.37 Turkish Lira (TL), and beyond 240 kWh, it costs 2.06 TL (1 kW equals 1000 watts).

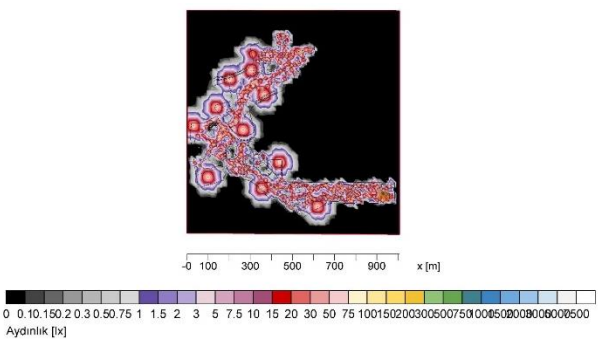

Figure 7. Settlement and Illuminance Levels Map of the Proposed Lighting Project

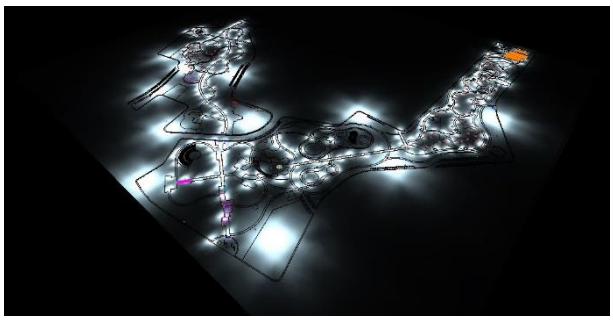

Figure 8. Light Intensity Map of the Proposal Lighting Project

Table 1. Current and Proposed Lighting Map Total Power Outputs

	Calculation Algorithm	Maintenance Factor	Total Luminous Flux (lm)	Total Power (W)	Total Power in Area
Available Lighting Project	Average indirect part	0.80	7529092.00 lm	96005.0 W	0.09 W/m² (1.77 W/m²/100lx)
Suggestion Lighting Project	Average indirect part	0.80	5167812.00 lm	27630.0 W	0.03 W/m² (0.87 W/m²/100lx)

Therefore, the earnings for August were calculated as 33 883 000 watts (or 33 883 kW) multiplied by 2.06 TL, which amounts to 69 712.98 TL. In light of this result, the 1-month revenue generated by a lighting application developed in compliance with energy efficiency and standards is approximately 69 712.98 TL. This highlights the significance of preparing the lighting design during the project phase in alignment with energy efficiency guidelines and standards, which can help prevent unnecessary expenditures and energy losses.

Conclusion and Suggestions

Urban lighting design should functionally meet the sense of sight, which is one of the most basic needs of people at night, and create a sense of visual comfort and security for the people of the city. In terms of aesthetics, it enables the citizens to benefit from visual and aesthetic values in terms of urban memory and silhouette by making urban elements visible and understanding at night as well as during the day. The use of urban areas in the evening and at night as well as during the day depends on the security it creates. At this point, the level of illumination should be at a sufficient level in order to ensure the security of the park and to perceive the park in the best way. This lighting level is set out within the scope of the project. However, within the scope of the thesis, it has been observed that energy saving and cost remain in the background in the lighting design and applications made for the park.

It has been determined that the illuminance levels provided by the currently used lightings are at a higher level than the proposal map, and it has been observed that there are negative situations in terms of energy efficiency. As a result of the cost calculation of energy losses, it has been revealed that there are significant expenditures for local governments.

The study investigated the compliance of Konya Butterfly Valley Park with park lighting criteria and standards, revealing that the landscaping areas were not illuminated in accordance with the specified standards and criteria. Negative outcomes in terms of user and visual comfort were observed not only during the design and implementation phases but also in the usage phase. When comparing the existing lighting project with the proposed lighting project, approximately a 1/3 energy savings and cost savings were achieved with the proposed lighting project.

For the Butterfly Valley Park (Konya), first of all, the extra lighting elements should be removed, and the values for the brightness levels should be increased in the areas that we call blind spots and which create a feeling of insecurity. In some areas, the illuminance levels should be increased, and while doing this, attention should be paid to obtaining the appropriate quality of light. While making these applications, attention should be paid to energy efficiency, and LED luminaires that are suitable for today's modern age and consume less energy should be used. In urban design and applications to be made, cooperation with local governments should be done and regulations, specifications, etc. The use of energy-saving luminaires with high efficiency should be expanded in documents.

In the Butterfly Valley Park, there are security-related problems in areas that are not illuminated. In order to solve this trust problem and to create a suitable design, the number of lighting elements should be increased where necessary. In this way, the areas remaining in the blind spot should be illuminated. Square in the park, etc. Attention should be paid to the illumination levels of meeting areas, playgrounds used by parents and children, and sports fields in accordance with the standards, and the lighting design should be made in the most appropriate way and the necessary lighting elements should be used. Since safety is one of the most important issues in children's playgrounds, lighting with 250W luminaires on 12-meter poles with high IP feature will be appropriate in terms of design. In order to avoid blind spots, the luminous intensity should be between 15-50 lux. Pedestrian, walking and bicycle paths, which are the areas most frequently used by park users, should also be adequately illuminated. The entrance, pedestrian and bicycle paths should be less than 5-10 lux illuminated. It is very important that the areas they mostly

use are bright so that visitors feel safe in the park. Some errors were observed in the lighting of the plant materials in the Butterfly Valley Park. These vegetal areas should be illuminated from top to bottom in a way that does not reflect the eye, while also adding visuality to the foreground. If tree lighting is done to create a focal point and emphasis, positive results will emerge in the park in terms of aesthetics. In addition to all these, considering the ecological condition of the trees, high voltage lamps should not be used. Therefore, it should be arranged so that the maximum luminous intensity for trees is 5 lux. Sitting areas, which are another frequently used area, should be illuminated with a luminous intensity of 15-20 lux so that visual comfort can be provided to the visitors who spend time sitting. In the lighting design to be made, besides the amount of light of the luminaire, its angle, spread and aesthetic values should be taken into consideration.

In the existing lighting design of the park, a special lighting design for the entrance could not be found. Therefore, a suitable entrance lighting design should be made and entrances should be emphasized. Since the park has entrances and exits to be zero to the street, lighting design should be done considering the vehicles passing through the street. When the entrances of the park are designed with appropriate lighting, it will be more comfortable to perceive and will separate the park from other urban areas. In addition, the entrance-exit lighting is very important in terms of guiding the visitors who come to the park at night. In the current design, attention should be paid to the positions of the lighting elements intertwined with the plants during the application and design phases, and the project phases should proceed in sync.

In order for the lighting elements in the park to have a positive effect in terms of visual, aesthetic and functional, their maintenance should be done regularly and new ones should be replaced with damaged, broken or irreparable elements. Lighting elements that can be easily mounted and intervened in case of any malfunction should be preferred during the design phase.

In the design process of lighting elements, lighting master plans prepared in line with urban analysis should be used. All decisions should be taken on the basis of basic urban data obtained in line with the plan, such as the region of the urban area where the element will be used, its location in the city, physical environmental conditions and areas of use, the location of the lighting elements in the area, their detection forms, numbers, sizes, etc. data should be evaluated.

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Analytical Hierarchy Process for the Selection of a Square: The Case Study of Konya City

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ABSTRACT

Rapid population growth, industrial and technological development, and improvement in the social and economical conditions of people have increased their need for socializing, gathering, and relaxing with various recreational activities and mutual communications. The sustainable development of social life has increased the importance of squares as public spaces, which brings the citizens together for cultural, commercial, and political purposes; thus, giving an identity to the city and becoming the focal point of urban life. The selection of an area as a square is based on certain criteria. The decisions regarding the choice of the location and their use as squares must be per the internationally accepted criteria. We studied four squares, namely Mevlana Square with historical background, Hükümet Square, Anıt Square, and Kılıçarslan City Square with a high demand for social events. The squares are considered to be important and comprehensive titles for evaluating their comparative functions under the selection criteria of visuality, functionality, and accessibility. The Analytical Hierarchy Process (AHP) method was used to determine the importance of the selection criteria of squares in a survey with participants for solving the problem and selecting the best square according to these criteria. By performing the AHP analysis, we found that the most preferred square by the participants was the Mevlana Square with a preference rate of 58.68%, and the most preferred criterion was "visuality" with a preference rate of 64.5%. In this study, we aimed to determine the characteristics of a preferable square to improve the existing squares and to contribute to the stages of a new square design, planning, and implementation.

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Introduction

People have needed each other for various purposes and have come together throughout history. The increase and diversification of human needs, great changes in social and economic conditions with the rapid population growth, developing industry and technology have led to an increase in the needs of people such as spending time with each other, socializing, gathering, relieving stress with various recreational activities and mutual communication, and getting out of the psychology of loneliness.

From the past to the present, squares have been the focus of urban life, which was the first to form in cities, and as the most widely and effectively used urban open space element of urban life, revealing the identity and personality of the city. When the squares that always serve the public in various aspects are examined, it is seen that they undertake one or more functions such as religious, political, recreational, commercial, cultural, and gathering (Özer and Ayten, 2005).

Squares are the most used public spaces in urban life where the physical environment interacts. Spaces have the characteristics of 'centres' where people come together for the development and maintenance of social life and for commercial, cultural, and political purposes (Demirel, 2008; Güngör et al., 2019).

Squares, which are among the urban open spaces, are the most significant factors in establishing communication between society and individuals, facilitating social interactions, and strengthening social awareness. Spending time with others and engaging in interactions positively influence personal development and psychology (Erdönmez and Akı, 2005).

Squares are spaces that cater to users' rest, entertainment, spiritual, and physical needs, fostering and strengthening communication among people, ultimately constituting and enlivening social life (Altınçekiç and Kart, 2001).

Squares have brought people together primarily for commercial purposes and provided opportunities for various activities (Yamaçlı, 1997).

Throughout history, squares have hosted various activities such as rallies, celebrations, markets, executions, demonstrations, and parades, serving multiple functions simultaneously. Surrounding the square, there have been various structures at different times, such as churches, residences, universities, and printing houses, which have influenced the square's diverse services (Öztaşkın, 2008; Sayın and Çorbacı 2019).

If an area serves different functions, all these functions should be accommodated without hindering each other in practice (Kürkçüoğlu, 2009).

To this end, it is necessary to look at the squares from the eyes of the people who use them and to design accordingly. Squares where many different social groups take place should be defined in line with the needs and expectations of the people.

The function or functions of a square are very important in terms of the vitality and visual appeal of the space. In terms of usability, squares should host various activities to attract people (Sertkaya and Çolak, 2011;Çorbacı and Ertekin 2017). Squares built to be functional should have an impressive and unifying feature (Marcus, 1998; Demirel, 2008). In this context, it is of great importance for the squares to be high quality and preferable areas to plan the squares correctly, to make a strong functional and aesthetic design and to ensure its continuity.

As the squares are spatially formed in the historical process, some areas can be arranged as new squares according to the requirements. In this context, the aim of the research is to determine the criteria groups and importance levels that will be used as a basis in the evaluation of the square functions of the areas that are used as squares in the city center of Konya and will be planned to be used for this purpose by the local governments, and to compare the squares that are the subject of the research.

It is necessary to define urban squares' qualities and to apply these qualities in existing areas and new designs for the realization of human use to the required extent and contribution to urban life. The important qualities of squares that have such an important place in people's lives should be determined and brought to the fore. For this purpose squares such as Mevlana Square, Hükümet Square, Kılıçarslan City Square and Anıt Square, that serve a large user group with different usage purposes and located within the borders of Konya which is the largest city of Turkey, have been selected. The visual and functional qualities of these squares and the parameters that may be effective on these criteria were determined, and a survey to be carried out with the help of these parameters was evaluated by AHP method.

In this study, AHP, which is a decision-making model based on priority ranking that emerged with paired comparisons of several elements or factors depending on certain criteria, was applied to the square options in the city center of Konya. The usability of the model in the comparison of city square options in the context of urban design has been tried. In the evaluation of city square options specific to the research area, findings that will shed light on the projects and practices to be carried out by local governments have been reached. Principles and suggestions that can form the basis for similar research, projects, and practices to be carried out in different cities, areas, and places were put forth.

Material and Method

The main materials of the research are Mevlana Square, Hükümet Square, Anıt Square and Kılıçarslan City Square, which are in the city center of Konya, at the center of the city's main transportation network, where gathering, resting and some recreational activities are intensely carried out, thus having a more user population and a diverse user profile.

Pairwise comparisons were used to evaluate the importance levels of square usage preferences.

Figure 1. Konya city and study areas

It is aimed to rank the importance level of the criteria of being a square in the example of the city of Konya and to rank the squares according to these criteria.

The setup of the research, which aims to prioritize and rank the options of Mevlana Square, Hükümet Square, Anıt Square and Kılıçarslan City Square with the help of the Analytical Hierarchy Process method, consists of five basic stages such as definition of the problem, model the problem as a hierarchy, evaluate the hierarchy, establish priorities, and the final decision.

Definition of the Problem: There are two main purposes of the study, which is made to compare the square options with the AHP method in Konya city center example. The first of these is the ordering of the importance level of the criteria of being a square, and the second is the ordering of the squares according to these criteria.

Model the Problem as a Hierarchy: The first step of the method applied in the study is the hierarchical structuring of the decision problem. At this stage, it is aimed to divide the decision-making problem into sub-items and to create a model that shows the relationships between these elements.

The levels and elements in the hierarchy are as follows:

Level 1: Purpose; Comparison of the square options with the AHP method in the example of Konya city center and the importance level of the criteria of being a square.

Level 2: Criteria; Visuality, functionality and accessibility criteria were determined by the synthesis of the functions, qualities, and quality criteria of the squares, which are public open spaces.

Level 3: Alternatives; Mevlana Square, Hükümet Square, Anıt Square and Kılıçarslan City Square.

Evaluate the Hierarchy: The user evaluation form, which was created according to the problem hierarchy, was prepared for the people who use the squares, for the user's opinion needed in the process of relative evaluation of different alternatives and the selection of the importance levels of the criteria most suitable for the purpose. It was conducted with 452 people visited these squares from different gender, age, education level and occupational groups to ensure a certain level of consistency in the problem solving and comparative judgments. The comparison of square options with the AHP method and the pairwise comparison matrices showing the judgments about the importance level of the criteria for being a square were prepared according to the user evaluation results. Final user preferences were determined by geometric averaging on the comparison data. The data obtained from the AHP rating scale were converted into a pairwise comparison matrix.

Establish Priorities: After the participants' decision problem, criteria and alternatives were revealed, they first determined the importance of the criteria with respect to each other by pairwise comparisons, and then the importance levels of all the options for each criterion by pairwise comparisons. Common values should be obtained by taking the geometric mean to make an evaluation with the common judgments of the participants.

In the second stage of the model, the participants determined the importance levels of four square alternatives by pairwise comparisons according to each criterion.

Final Decision: In the evaluation made to compare the square options with the AHP method in the Konya city center example and to determine the importance level of the criteria for being a square, criteria and alternatives were taken into consideration. The user opinions were entered into a database in Microsoft Excel. The geometric mean and consistency ratios of these data were calculated for each of the pairwise comparisons. The relative importance value and the criteria according to the purpose, the alternatives according to the criteria are listed. The final decision, reflecting the opinions of the users who know Konya city center closely, was made according to the findings obtained from the solution of the problem, multiplying the total composite relative importance value with the weights of the criteria and the weights assigned to the alternatives according to the criteria.

Results

To compare the squares present in the center of Konya city by the AHP method and to evaluate the importance of square criteria, three criteria such as visuality, functionality, and accessibility of four squares such as Mevlana Square, Hükümet Square, Anıt Square, and Kılıçarslan City Square in the city center of Konya were examined. The analysis was performed by pairwise comparison. The relative important value, the criteria according to the purpose, and the alternatives according to the criteria are listed.

According to the participants in the survey, when the degree of importance of the three criteria is evaluated, the visuality criterion with a preference rate of 64.5% was in the first place, the functionality criterion with a preference rate of 26.5% was in the second place, and the accessibility criterion with a preference rate of 9% was in the third place (Table 1).

Table 1. Decision criteria weight scores

Decision Criteria	Weight Rating
Visuality	0.645
Functionality	0.265
Accessibility	0.090

This study is important to determine the best alternative criteria and present developmental strategies for each square. The AHP analysis results showed that the Mevlana Square is the most preferred square based on all three criteria. The weightage of the criteria for square selection by the participants is presented in Table 1. The participants preferred the Mevlana square with a preference rate of 60% in terms of visuality, 57.3% in terms of functionality, and 52.9% in terms of accessibility. The Mevlana square had the highest values of all criteria. Accessibility was the most preferred criterion with a preference rate of 26.6% in Hükümet Square, 13.9% in Anıt Square, and 6.4% in Kılıçarslan City Square (Figure 2).

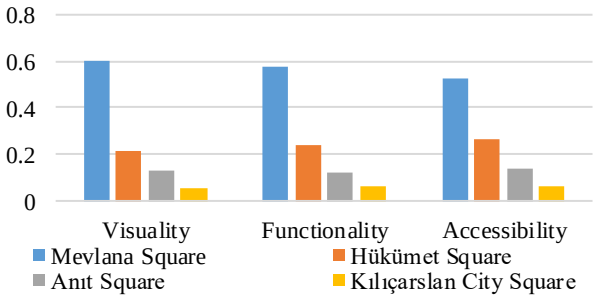

Figure 2. Ranking of alternatives for each criterion

The final decision reflects the opinions of the users who know Konya city center closely and visit these squares. By solving the problem, the total composite relative important value was obtained by multiplying the weights of the criteria and the weights assigned to the alternatives according to the criteria (Table 3). Mevlâna Square stands out as having superior visual aesthetics; however, its functionality and accessibility are evaluated as relatively lower compared to the other squares. On the other hand, Hükümet Square emerges as a robust alternative in terms of functionality, albeit with less pronounced visual appeal compared to the other squares. Anıt Square holds an advantageous position with regard to accessibility considerations but garners lower scores in other critical criteria. Finally, Kılıçarslan City Square exhibits an overall inferior performance when compared to the other squares.

Table 3. General Synthesis Table

Decision Criteria	Weight Score	Criterion Points of Squares			
		Mevlâna Square	Hükümet Square	Anıt Square	Kılıçarslan City Square
Visuality	0.645	0.600	0.213	0.132	0,056
Functionality	0.265	0.574	0.241	0.125	0,060
Accessibility	0.090	0.530	0.267	0.139	0,064
The final decision		0.587	0.408	0.095	0.024
Arrangement		1	2	3	4

Conclusion

The scope of the research included four squares such as Mevlana Square, Hükümet Square, Anıt Square, and Kılıçarslan City Square and three selection criteria such as visuality, functionality, and accessibility.

In this study, in addition to choosing the best square in terms of selection criteria, the importance of the criteria of a square was also evaluated based on the results of AHP analysis. The results also presented the developmental strategies for the squares present in the Konya city center. According to the evaluation results of the Mevlana Square, Hükümet Square, Anıt Square, and Kılıçarslan City Square and the alternatives examined in the study, we found that the visuality criteria was most preferred by the users. The Kılıçarslan City Square and the accessibility criteria were least preferred as per the rankings by the participants of the survey. As the population of the city increased, the need for housing increased, which increased the pace of construction in the city, and the growth of the city became inevitable. The development in technology, industrialization, and increased welfare of the people worldwide has increased the transportation networks and the use of public and private vehicles. Thus, the citizens can reach their desired destination easily. We assume that these factors are the possible reason for the lowest ranking of the accessibility criteria by the participants.

When the binary comparison of the squares and the criteria were considered one at a time, we concluded that the Mevlana Square was the most preferred square among the four squares based on all three criteria. Mevlana Celaleddin Rumi is popular for his unconditional tolerance and the philosophy of humanism worldwide and his tomb is situated at the Mevlana square. Therefore, the Mevlana square is the most visually preferred square by people, which is shown by our data. The Mevlana square is easily accessible by all types of transportations and provides an opportunity for gatherings and religious visits.

The Hükümet Square is the second most preferred square after Mevlana Square. As the square is located at the focal point and crossing point of the city, its accessibility rate is high. In the past, ceremonies such as enthronement were held in Hükümet Square. Currently, the square is used for administrative purposes as it is situated next to the government building and also for commercial purposes as it is situated next to the Historical Bedesten Bazaar. The proximity of the Şems tomb and the presence of the Şerafettin Mosque nearby for religious visits have affected the value of the functional criteria because of the square's versatile usability. The plants near Hükümet Square have to be increased to improvise the design and to increase its relationship with the structures that increase the urban identity in its vicinity.

In the Anıt Square, all three criteria showed similar results. The Anıt square is smaller in size than the other squares. The square is not large enough for public gatherings; therefore, its usage is low. The Atatürk Monument that was built in 1926 has given the name, image, and identity to the square, which is situated at the junction of some of the main streets of the city. Various ceremonies are hosted in the square, especially on national holidays such as 10th November, 23rd April, and 29th October every year. The existence and usability of the reinforcement elements in and around the square should be improved.

The Kılıçarslan City Square is observed to be the least preferred square by the users in terms of all criteria and general ratio. The Kılıçarslan City Square has been built recently and is close to Alaaddin Hill. Therefore, compared with the other three squares, the Kılıçarslan City Square does not have any historical background. The lack of greenery and equipment in and around the square is another reason for its least popularity among the users. Therefore, the number of plants near the square should be increased, which can enhance the design of the area. The existence and usability of infrastructure in plant applications should be increased. As the square can respond functionally to functions and changes that may occur later, structures that will ensure its relationship with the social and cultural facilities in its vicinity should be created.

We conclude that the AHP method, which is one of the most commonly used analytical and multi-purpose methods, can be used successfully for finding solutions on such hierarchical models. The method has advantages by providing a consistency criterion for evaluations in comparison charts, allowing cross-checking, and providing effective solutions to similar problems compared with other methods. The AHP method is considered one of the best methods that can select the best alternative criteria. By identifying the importance levels of the criteria, it can be possible to review and develop the low criteria as well.

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Detection of *in silico* SSR Markers Specific to Uzun and Kırmızı Cultivars in Pistachio

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ABSTRACT

In the current paper, it was aimed to detect the SSR markers that can be used in the prevention of confusion that may occur in breeding or nurseries, and directly genetically separating Uzun and Kırmızı pistachio cultivars from other commercial cultivars. A total of genotypes of 16 *Pistacia vera* species, one *P. atlantica*, one *P. eurycarpa* and two *P. terebinthus* species were obtained from the farmer's orchard in Nizip district of Gaziantep province for genetic characterization. Genetic diversity and clustering analyzes were performed with UPGMA (Unweighted Pair Group Method with Arithmetic Average) and STRUCTURE 2.3.4 programs using the scored SSR loci. Genetic relationship and population structure of genotypes were defined using common and distinct polymorphic PCR fragments. Cultivar-specific markers to be used in identifying and distinguishing the genetic structure of Uzun and Kırmızı cultivars were carried out in the current research. CUPOhBa2127 marker has the highest allele number (Na=10). In addition, 11 out of 25 SSR markers were explained as cultivar-specific SSRs that can distinguish Uzun and Kırmızı cultivars. These markers can be used directly by breeders and geneticists without any preliminary screnning of the markers. A quite serious providence will be achieved in the cost and time that will occur with the preliminary analysis, and thus, the confusion that may occur in large scale orchard establishments or nurseries will be reduced to pretty low levels with DNA analysis.

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Introduction

Pistacia vera L. takes place in the genus *Pistacia* of the Anacardiaceae family, and there are at least 11 species in the genus *Pistacia* (Kafkas, 2006a). *P. vera* is the only edible *Pistacia* species and it has commercially importance. *Pistacia* species are pollinated by the wind and they have dioecious inflorescences nature, except for monoecious genotypes (Kafkas et al., 2000). The chromosome number of the pistachio is 2n=30 (Kafkas, 2022). The origin of the pistachio defines as from Central Asia and spread from Southern Europe to China, and it has existed in the United States, Australia and the Mediterranean region (Kafkas, 2006b). Among 30°-45° in the south-north parallels are pretty suitable for cultivation which are accepted as microclimate conditions such as in the Northern Hemisphere geographical regions (Tunalıoğlu and Taşkaya, 2003). The amount of world production of pistachio reached 1,375,770 tons in 2022, and the main producing countries were Iran, the USA, and Turkey (Faostat, 2022).

The previous molecular diversity papers in plants have been carried out in many plant species with different molecular methods. The molecular markers enabled the polymorphism to be detected effectively based on DNA at an early phase of plant growing for marker assisted selection in terms of agronomic important properties (Orman et al., 2020; Paizila et al., 2022). So far, they have been used in genetic diversity, mapping and marker assisted breeding. Several molecular markers have been developed and used in distinct plant species (Güney et al., 2018; Khodaeiaminjan et al., 2018; Karcı et al., 2022). Simple Sequence Repeats (SSRs) are the PCR-based method, and it is one of the most preferable technic by geneticist and breeders for characterization studies of cultivars and genotypes due to its codominant inheritance nature, spread throughout the whole genome, high polymorphism and reproducibility, and high transferability rate (Kafkas, 2019; Karcı et al., 2022). The performed papers in apricot (Hormaza, 2002), pear (Fan et al., 2013), almond (Esgandaripirmorad et al., 2022), pistachio (Karcı

et al., 2022) and walnut (Güney et al., 2022) were main examples. SSRs are pretty beneficial technic for evaluation of genetic relationships among the cultivars, and it is an essential tool for identification of the genetic diversity and genomic nature in pistachio breeding programs for characterization of the germplasm. The usage of SSRs to identify the genetic relationship between pistachio cultivars or landraces is pretty reliable tool, and they play a significant role in overcome to many issues.

The papers related to molecular genetic of pistachio have been published from last three decades to today with several marker systems such as RAPD, AFLP, ISSR and SSR, respectively (Hormaza et al., 1994; Kafkas et al., 2006; Kolahi-Zonoozi et al., 2014; Karcı et al., 2022). The first investigation in pistachio DNAs was performed using the RAPD technique (Hormaza et al., 1994). In the following years, Kafkas et al. (2006) published an article related to the genetic relationship of pistachio germplasm by using RAPD, AFLP, and ISSR markers. In addition, Khadavi et al., (2018) carried out genetic diversity analysis with SSR markers developed by Topçu et al. (2016) using only Iranian pistachio genotypes. In a recent report carried out by Karcı et al. (2022), the large-scale pistachio genetic resources were characterized by SSR markers.

Here, genetic diversity status of pistachio genotypes sampled from Nizip district of Gaziantep province were identified, and SSR markers specific to Uzun and Kırmızı cultivars were mined from polymorphic *in silico* SSR markers.

Materials and Methods

Plant material and DNA extraction

A total of 16 pistachios (*P. vera*) cultivars and genotypes, one *P. atlantica*, one *P. eurycarpa* genotypes and two *P. terebinthus* genotypes were used in the present research. Pistachio plant samples were taken from Nizip district in Gaziantep province. Samples of wild species were used to identify the genetic dissimilarities of *P. vera* samples to other *Pistacia* species most abundant in Turkey.

Total genomic DNA was isolated from fresh young leaves by the CTAB method described by Doyle and Doyle (1987) with some modifications (Kafkas et al. 2006). Qubit Fluorometer (Invitrogen) was used to quantify the isolated DNAs, followed by diluting them to 10 ng/µl for SSR-PCR reactions, and then the samples were stored at -20°C for further analysis.

SSR-PCR reactions

Totally, 52 most polymorphic SSR primer pairs were selected from developed by Khodaeiaminjan et al. (2018), and they were screened (Table 1). Of the 52 primers screened, 25 scoreable and polymorphic SSRs were selected according to their polymorphism levels.

All SSR-PCR reactions were done based on a three primer strategy according to Scheulke (2000) with minor modifications. A total volume of 12.5 µl containing 10 ng DNA, 20 mM $(NH_4)_2SO_4$, 75 mM Tris–HCl (pH 8.8), 0.01% Tween 20, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 10 nM M13 tailed forward primer at the 5′ end, 200 nM reverse primer, 200 nM universal M13 tail primer (5′-TGTAAAACGACGGCCAGT-3′) labeled with FAM, VIC, NED or PET dye, and 0.6 U of Taq DNA polymerase were used for each reaction. PCR amplifications were done in two consecutive steps. The first step included initial denaturation at 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 60 s. The second step involved 10 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 60 s, and a final extension at 72°C for 5 min. When the PCRs were completed, the reactions were subjected to denaturation for capillary electrophoresis in an ABI 3130xl genetic analyzer [Applied Biosystems Inc., Foster City, CA, USA (ABI)] using a 36-cm capillary array with POP7 as the matrix (ABI). Then samples were denatured by mixing 1.0 µl (in 6-FAM and VIC labeled primers) or 1.5 µl (in NED and PET labeled primers) of the amplified product, 0.3 µl of the size standard and 9.7 µl of Hi-Di formamide. The ABI data collection software 3.0 was used for resolving the fragments, and then SSR fragment analysis was done using the GeneMapper 4.0 (Applied Biosystems Inc.).

Data analysis

After capillary electrophoresis of the SSR loci, effective number of alleles (*Ne*), expected heterozygosity (*He*), the number of alleles per locus (*Na*), and observed heterozygosity (*Ho*) were calculated using the GenAlEx version 6.5 program (Peakall and Smouse, 2012). Polymorphism information contents (*PIC*) for the SSR loci were calculated using PowerMarker software version 3.25 (Liu and Muse, 2005).

The UPGMA (Unweighted Pair Group Method with Arithmetic Average) based dendrogram analysis was conducted in MEGA*X* software (Kumar et al. 2018). Population structure and identification of admixed individuals was performed using the model-based software program, STRUCTURE 2.3.4 (Pritchard et al. 2000). In this model, a number of populations (K) are considered to be available, which each of them is characterized by a set of allele frequencies at each locus. Individuals in the sample are given to populations (clusters), or jointly to more populations if their cultivars indicate that they are admixed. Ln P (D) values (logarithm probability for each K) were applied to determine the Delta K indicating the probable population number. The term of Delta K is calculated by the change ratio of logarithm probability ($\Delta K=2$ to $\Delta K=5$). In the diagram, the highest K of Delta K confers the information about the probable population number.

Results and Discussion

Polymorphism degrees of SSR markers

Totally, 25 SSR markers were scored, and PCR fragments were recorded, and genetic diversity analysis was performed on a total of 20 genotypes (Table 1). The produced 125 alleles all genotypes were used in the current research, and the number of alleles were varied from 2.00 to 10.00 alleles, while the average number of alleles was 5.00. The highest number of alleles was calculated at the CUPOhBa2127 locus (Na=10). The number of effective alleles (Ne) was identified from 1.13 (CUPSiPa2066) to 3.86 (CUPOhBa2127 and CUPSiPa3487), and average effective number of alleles was 2.23. The mean observed heterozygosity (Ho) value was 0.46, and it ranged from to

0.00 to 1.00. The highest observed heterozygosity (Ho) was found at the CUPSiOh510 locus. The mean He value was explained as 0.49, and the highest expected heterozygosity (0.74) was detected from the CUPOhBa2127 and CUPSiPa3487 markers. The average of the polymorphism information content (PIC) was calculated as 0.45, and was found between 0.11 (CUPSiPa2066) and 0.70 (CUPOhBa2127 and CUPSiPa3487) (Table 1).

Various molecular papers have been performed related to genetic variation and relationships of pistachios and other *Pistacia* genotypes with SSRs (Zaloglu et al., 2015; Khodaeiaminjan et al., 2018; Karcı et al., 2022). The first SSR development research was reported by Ahmad et al. (2003, 2005) using a limited number of pistachio cultivars. Since there are not enough SSR markers to define genetic diversity of the pistachio cultivars, Kafkas et al. (2006) performed a research using AFLF, ISSR and RAPD markers in large scale pistachio cultivar collection. In addition, Iranian pistachio cultivars has recently been characterized developed by Topçu et al. (2016) (Khadivi et al., 2018).

Table 1. Forward and reverse sequences of the markers, SSR motifs, Na, Ne, Ho, He and PIC values of polymorphic *in silico* SSR markers

No	Markers	Forward 5'-3'/Reverse 3'-5'	The motifs of the repeats	Na	Ne	Ho	He	PIC
1	CUPOhBa2127	F:TGGAAGAACAAGTGAGGAGCA R:GTTGAGGAAGGAATGGAGGTC	-	10	3.86	0.8	0.74	0.7
2	CUPSiOh3876	F:CCTATTTCCCCTTACTTCTTCCA R:TCAAACCTTAGTGAAGGGCATT	$(TC)_{21}$	9	3.4	0.6	0.71	0.67
3	CUPSiOh3348	F:CTTGAAAATTGTTTTGCAGT R:CAGACTGAAAGTTTAAGATTGA	-	8	2.22	0.44	0.55	0.53
4	CUPSiOh3646	F:ATACACCCGTGTGAAATGCAA R:GGCTGGTAGTCTGGTGCTTT	$(TC)_{15}$	4	3.23	0.59	0.69	0.64
5	CUPSiPa694	F:TGTTGTATGCAATACCCTAGATT R:TCATGTGTATTCATTGGTCGAT	$(AAT)_{10}$	7	1.93	0.3	0.48	0.46
6	CUPBaPa992	F:CGCAAAGAGTTTTTCAAAGAGG R:TGGTTTCAAATACCGAAAAACA	$(CT)_{12}$	6	1.73	0.25	0.42	0.4
7	CUPSiPa3487	F:TGAGAGTCGTGTAAGGGCTTC R:CTGTTTAAGGAACGGAAAGG	-	7	3.86	0.8	0.74	0.7
8	CUPSiOh2834	F:GCGCTGTAAATCCAAGAAAAC R:TGTTCGTTGTTGCCTTTCTTT	$(AG)_{16}$	3	1.59	0	0.37	0.34
9	CUPSiOh4004	F:TGGGGCTAAAAATCACTTCAC R:TTGCAAAATGAGTTTGGAGGT	$(TG)_{13}$	3	2.15	0.69	0.54	0.43
10	CUPSiOh2178	F:CCAGAATTTGTTGGAAGTTGC R:TTATCTCACATGAGGCAAAAT	-	6	1.92	0.18	0.48	0.46
11	CUPSiPa1583	F:GAGAAGTAAAAGAAGGGACGGTTA R:TTTCTTCCATAATCAATCCGACT	$(GA)_{11}$	7	1.79	0.32	0.44	0.42
12	CUPSiOh3920	F:GAAGGGAAGGAGAGAACGATG R:GAAAAACAACAAAGCGACGAC	$(GAT)_4CTATTTATCGCAG(AGGCTC)_4$	6	3.8	0.53	0.74	0.69
13	CUPSiPa1904	F:ATCCAGAATCCAAGGGAAGAA R:CAATATGGCCAGACTCAGCAT	-	5	1.39	0.19	0.28	0.27
14	CUPSiOh4419	F:CAAACTAAATGTGGTGCATTATCAA R:GCTTTGCTAGATAAAAATACCCAGA	$(TAT)_{10}$	3	1.95	0.73	0.49	0.4
15	CUPSiOh2995	F:TGCATTTTCAGCTTCAATGTC R:TGACCCCTCTTCTCTTTCACC	$(AAG)_8$	4	1.21	0.19	0.18	0.17
16	CUPBaPa2462	F:TCAAGCTTTCTTGTCATCACCT R:GCTCAACACTTGGTTTTGTCTTT	$(TAA)_9$	4	1.37	0.3	0.27	0.26
17	CUPSiOh3903	F:GAATATACATGGTGGACCCTCA R:GAATAGGGTTCGTACCTGCAA	$(AAT)_{10}$	3	2.11	0.44	0.53	0.44
18	CUPSiOh3994	F:AGGTCGCAGATAACGAGTTGA R:CACCTGTTGAACAATAGGCTCA	$(AG)_{14}$	5	2.84	0.93	0.65	0.58
19	CUPSiOh510	F:CATTTTTCATTTTGGAGCTGAA R:ATTGCAGGAAAACAAGCAAAG	-	5	2.91	1	0.66	0.6
20	CUPOhBa2087	F:CTGCAATTTATGAAAAGTTGTTCTC R:GCTTTGAGCTTCTTTCACAACTC	$(ATT)_8$	4	1.46	0.21	0.31	0.3
21	CUPOhBa2356	F:GCATGCGTGCTGGATTTATAC R:TTTGACGACTTTCCCACACTT	$(AG)_{18}$	3	1.46	0.18	0.31	0.29
22	CUPSiPa2066	F:CAGCTCCCACTAGGTTTGTGT R:ACAATCTCACAACAAACAAAGAACA	$(TTGTTA)_6$	2	1.13	0.13	0.12	0.11
23	CUPSiOh2340	F:GACTACTGTGCCCACATGACA R:GGATCGTCAGAGAAGACGTTG	-	3	1.66	0	0.4	0.35
24	CUPSiOh1325	F:TTTTCTTTGATCTTTCTACCGCTAC R:TGAGCAAACAATACAGTTGAATCC	$(ATTT)_8$	6	2.75	0.92	0.64	0.57
25	CUPSiPa912	F:TGCAGTGAGTAGGAAGTTTGGA R:AGCGAACAAGAGAACGAACAC	$(TGAGTG)_5$	2	1.95	0.83	0.49	0.37
Mean				5	2.23	0.46	0.49	0.45

Table 2. The fragments and frequencies of SSR markers specific to Uzun and Kırmızı pistachio cultivars

No	Markers	PCR fragments	The frequencies of the markers	Cultivars
1	CUPSiOh3876	142	0.275	Uzun
2	CUPSiOh3646	182	0.265	Uzun
3	CUPSiPa3487	113	0.025	Kırmızı
		115	0.250	Uzun
4	CUPSiOh2834	164	0.111	Kırmızı
		166	0.778	Uzun
5	CUPSiOh4419	159	0.033	Kırmızı
		192	0.333	Uzun
6	CUPSiOh2995	161	0.031	Kırmızı
7	CUPSiOh3903	145	0.344	Uzun

The mean allele values reported in previous SSR studies were 3.30 (Ahmad et al., 2003), 2.75 (Baghizadeh et al., 2010), 2.80 (Arabnezhad et al., 2011), 3.60 (Zaloglu et al., 2015), 4.2 (Khodaeiaminjan et al., 2018) and 2.73 (Karcı et al., 2020). In this paper, totally, 125 alleles were created with an average of 5.00 alleles per locus derived from 25 SSRs. In previous findings, the reasons of the differentiation of allele counts are low number of cultivars or genotypes, insufficient number of markers, and limited genetic variation among the individuals (Karcı et al., 2022). Despite the limited number of SSRs of present paper, the high average number of alleles was identified due to the high variation among the genotypes.

The mean polymorphism information content was calculated as 0.45, and it varied between and 0.11 and 070. The explained mean PIC in this study was found higher than 0.33 (Kolahi-Zonoozi et al., 2014) and 0.44 (Baghizadeh et al., 2010) and below 0.64 (Khadivi et al., 2018), respectively. The mean He and Ho values were determined as 0.49 and 0.46, respectively, although Khadivi et al. (2018) stated that these values were 0.22 and 0.44. And, Kolahi-Zonoozi et al. (2014) defined that those were 0.35 and 0.49. Khodaeiaminjan et al. (2018) reported that the average PIC, Ho and He values obtained from a total of 18 *P. vera* cultivars were 0.51, 0.53 and 0.56, respectively. A similar result of the He, Ho and PIC was calculated from 51 genic SSR markers designed from *P. vera*, and they were 0.40, 0.38 and 0.34, respectively (Karcı et al., 2020). On the other hand, Arabnezhad et al. (2011) calculated the mean Ho value as 0.64 from18 SSR markers fragments, while Baghizadeh et al. (2010) calculated the mean Ho value as 0.52 from four SSR markers. In addition, the mean He values performed by previous papers were found as 0.45 and 0.75, respectively (Arabnezhad et al., 2011; Baghizadeh et al., 2010). The waves of the genetic diversity results in the previous researches were based on the genetic variation of the populations, the scale of the characterized genotypes or cultivars and the number of screened primer pairs and their polymorphism levels. Thus, the polymorphism information content and the observed and expected heterozygosities resulted from a greater or lesser abundance of polymorphic SSRs, or a higher and less narrow rate of genetic variation in the population.

Identification of cultivar-specific SSR markers

A total of seven (7) primer pairs were generated 10 cultivar-specific genomic fragments in Uzun and Kırmızı pistachio cultivars, and allele frequencies ranged from 0.031 to 0.778. Of the cultivar-specific markers, CUPSiOh3876, CUPSiOh3646, CUPSiPa3487, CUPSiOh2834, CUPSiOh4419, CUPSiOh2995, and CUPSiOh3903 produced cultivar-specific alleles for Uzun pistachio cv., while CUPSiPa3487, CUPSiOh2834, CUPSiOh4419 and CUPSiOh2995 produced cultivar-specific alleles for Kırmızı pistachio cultivar.

The characterization of the accessions using SSR markers is a quite effective tool for early selection in different breeding programs (Ashkenazi et al., 2001). The identified cultivar-marker specific to Uzun and Kırmızı cultivars can be used by many breeders, geneticists and even farmers in pistachio breeding programs and nurseries. In addition, several SSR markers have been used in many previous studies; for example; detection of disease resistance alleles in rice (Melaku et al., 2018), triploid apples (Mazeikiene et al., 2019) and the sex determination of sugarcane (Pan et al., 2006). Therefore, a total of 10 cultivar specific markers that can separate Uzun and Kırmızı cultivars without any preliminary screening of the primer pairs can be beneficial for future pistachio breeding programs.

Detection of genetic similarities of the pistachio accessions

Phylogenetic analysis of 20 cultivars and genotypes was performed using 25 SSR primers amplified in all *Pistacia* accessions. *Pistacia* individuals were clustered in two main groups by UPGMA analysis (Figure 1). *P. vera* cultivars and genotypes were included in the first group in the dendrogram, while wild types of *Pistacia* species were included in the second group. Genetic dissimilarity coefficients among all cultivars ranged from 0.00 to 1.00. The highest genetic distance was detected between *P. vera* genotypes and wild accessions. According to the UPGMA analysis, Uzun cultivar was clustered with Kırmızı-1, Kırmızı-2, Pv-1, Pv-2, Pv-4 and Pv-10 genotypes, and the genetic difference coefficient was 0.00. Thus, these genotypes may be same genetic structure with Uzun cultivar. On the other hand, they separated from Kırmızı pistachio cultivar in early step.

Cluster analysis of 20 cultivars and genotypes was performed using 25 SSR loci with the STRUCTURE and STRUCTURE HARVESTER programs. The highest Delta K (ΔK) value was obtained at $\Delta K=2$ (Figure 2). $\Delta K=2$ indicates the possible population number of cultivars and genotypes. Therefore, accessions were divided into two main clusters, similar to the UPGMA analysis results. The genetic relationships of the genotypes were detected by both UPGMA and STRUCTURE analysis, and the results of both programs were found compatible with each other (Figure 2).

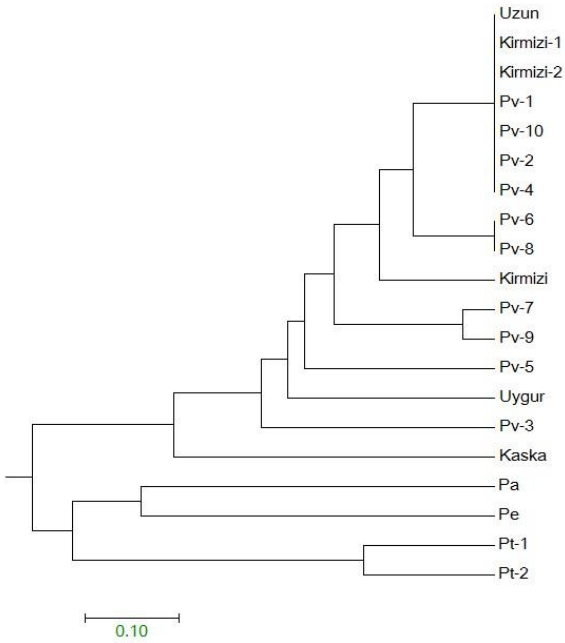

Figure 1. Dendrogram of UPGMA analysis of pistachio genotypes

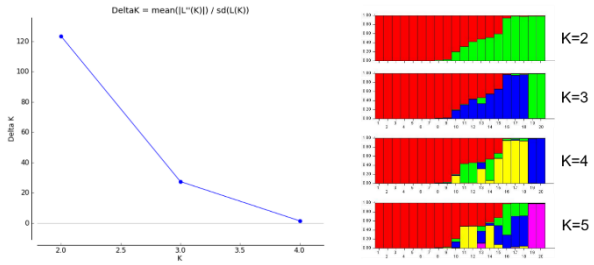

Figure 2. ΔK graph and K values of pistachio individuals consisted of the structure analysis

According to the SSR-based structural and UPGMA analysis clustering results, the original Kırmızı cultivar was clearly distinguished from the other Kırmızı-1 and Kırmızı-2 genotypes. However, many studies have reported that Kırmızı and Uzun cultivars were genetically close varieties (Khodaeiaminjan et al., 2018; Karcı et al., 2022). Similarly, Kaska and Uygur cultivars were reported by the same researchers to be close to wild *Pistacia* species, although they were included in *P. vera* cultivars. Similar results were obtained in both UPGMA and STRUCTURE programs in the current paper. The previous study performed by Kafkas et al. (2006) was carried out using ISSR, AFLP and RAPD markers, however these techniques have many disadvantages such as automation applicability, less reliability and reproducibility compared to the SSR marker technique. Therefore, although the results of the clustering were similar to their research; dominant marker systems are not practical for getting rapid results in the labs. In conclusion, the detected polymorphic and cultivar specific SSR markers may be preferred for geneticists and breeders to identify cultivars in the early stages of nursery and pistachio breeding studies. Thus, it can be saved for time and costs derived from extra laboratory processes, and prevention of mistakes based on the density of markers in the populations. Cultivar-specific SSRs can be used more accurately and quickly by geneticists and breeders studying *Pistacia* breeding.

Conclusion

DNA fingerprints of 20 genotypes belonging to *Pistacia* were identified by using 25 SSR markers and the accessions were characterized by two different genetic analysis methods. It has been proven once again that the SSR marker system is a pretty useful and efficient marker system in terms of the practical. In addition, it is quite significant to identify cultivars in breeding programs and nurseries in the early phase. In particular, the fact that Uzun and Kırmızı are the two most important cultivars preferred in the pistachio dessert industry due to their high aroma and green kernels, and the fact that these varieties can be genetically identified from other commercial cultivars or selections without the need for a preliminary screening analysis were important findings in terms of breeding. Cultivar-specific SSR markers presented can be also used in the identification of these two cultivars, without the need for long-term screening with many previously published SSR markers.

Declarations

Ethical Approval: This manuscript does not contain any studies with human participants or animals performed by the author.

Consent to Participate and Publish: The author reviewed and approved the final version for publication.

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Determination of Yield and Quality Parameters in Pickling Hot Peppers Grown under Different Water Stress Conditions

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ABSTRACT

Plants are exposed to various adverse environmental conditions throughout their growth period. In recent years, drought, which has occurred and necessitated different measures, ranks among these adverse conditions. At the same time, plants synthesize certain biochemical compounds in response to the adverse conditions they will encounter. These compounds not only strengthen the immune system but also provide resistance against various diseases, and they tend to increase under adverse environmental conditions that plants will face during cultivation. This study was conducted to determine the changes in yield and some biochemical components in pickling hot peppers (*Capsicum Annuum* L.) grown under different water stress conditions. Two different levels of water stress (%70 and %30 irrigation) were applied in addition to full irrigation (%100 irrigation). At the end of the research, while a yield of 269.42 g per plant was obtained in the control group (%100 irrigation), 150.14 g and 93.33 g of pickling hot peppers were harvested in each water stress treatment, respectively. Total phenolic compound levels increased with water stress; it was determined to be 0.827 $mg^{-1}g$ in the trial irrigated with full irrigation water, 1.170 $mg^{-1}g$ in plants exposed to mild water stress, and up to 1.536 $mg^{-1}g$ in the trial subjected to severe water stress. In addition, total flavonoids and antioxidant compound levels also increased with increasing water stress. The amounts of flavonoid compounds obtained from the trial groups were 0.146, 0.373, and 0.412 $mg^{-1}g$, respectively, while the antioxidant levels determined by the DPPH method increased in quantity with increasing water stress, similar to other biochemical compounds. According to these results, it was determined that the yield of pickling hot peppers decreased in the case of water shortage that the plants would face in cultivation, but there was an increase in some biochemical compounds.

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Introduction

Rising temperatures and reduced rainfall in recent decades have increased drought in regions within tropical and subtropical climate zones, negatively impacting food production in many areas (Erken, 2022). The increasing frequency and duration of drought worldwide can lead to the death of drought-sensitive plants causing a threat to future global food security (Naing and Kim, 2021). Considering your countries per capita annual water availability, Turkey is facing water scarcity. Given the adverse effects of factors such as climate change, environmental pollution, industrial development, and rapid population growth on water resources, it can be said that Turkey is expected to face significant water shortages in the future. Finding new water sources alone may not be sufficient to solve the problem; rather, ensuring the efficient use of water is essential. Efficient water use depends on changing irrigation practices and adopting new irrigation methods (Kanber et al., 2005).

Plants are the organisms most exposed to abiotic and biotic stress factors. Abiotic stress conditions such as drought, salinity, excessive rainfall, temperature, and cold directly affect plant growth and development. Depending on the intensity and duration of water stress, they can exhibit significant metabolic changes when confronted with drought conditions, which can regulate their life cycles significantly (Öztürk, 2015).

Pepper is a crop of significant importance for consumers, producers, and the processing industry, cultivated in various countries both in open fields and undercover (Duman et al., 2002). Taxonomically known as "capsicum" and commercially and scientifically referred to as "pepper," it is described as hot or chili pepper. Peppers belong to the *Solanaceae* family and have a sharp taste in food with a natural plant color. Moreover, due to their pharmaceutical content, they are also used in organic pest control by applying them in spray form on plants

(Sanatombi and Sharma, 2008). Hot peppers contain various phytochemicals, as well as vitamins A and C, phenolic compounds, flavonoids, and carotenoids. They are particularly known for their high vitamin C content and strong antioxidant levels (Yaldız and Özgüven, 2011).

In a field trial conducted in Bursa Province in 2019, the effects of four different drip irrigation water levels (S_{100}, S_{75}, S_{50}, S_{25}) on some yield components and water use efficiency of the Burkalem pepper variety were examined. The highest pepper yield was reported to be obtained from the irrigation levels where the crop coefficient was S_{100} (kc=1.00) and S75 (kc=0.75) (Yılmaz, 2022). In another study conducted under Çanakkale conditions, the effects of five different irrigation levels on plant fruit yield and some quality parameters were examined in the California Wonder pepper variety. It was stated that the highest yield was obtained when the crop coefficient was applied as k=0.75 and k=1.00 in the irrigation levels (Erken, 2004). According to the results of another study conducted on Jalapeno pepper (Capsicum annuum L.) with three different irrigation water levels (W1:%100, W2:%75, W3: %50), it was reported that with increasing water stress, stem diameter, fruit diameter, shoot length, shoot fresh weight, shoot dry weight, fruit length, fruit weight, leaf count, leaf thickness, and fruit count decreased, but root length, root fresh weight, root dry weight, and water-soluble dry matter content increased (Bilgin, 2019).

This study was conducted to determine the morphological differences and changes in the synthesis of antioxidant substances, phenolic compounds, flavonoids and proline levels in hot peppers exposed to drought throughout the cultivation period.

Materials and Methods

The research was conducted in the greenhouse of Çanakkale Onsekiz Mart University, located at 40° 08' north latitude and 28° 20' east longitude. The trials were carried out using short and thin peppers known as Hungarian hot peppers. Seedlings were planted on June 1, 2021, in 10-liter pots containing clayey loam soil with a pH of 7.25, EC of 0.50 $mS^{-1}cm$, 10.35% calcium content, and 2.88% organic matter content. In the experiment, which was designed according to a randomized complete block design, cultural practices were applied as necessary, and pickling hot peppers (*Capsicum annuum* L.) were grown until 27th of September. During the cultivation period, in addition to the control pots which were irrigated normally, two different water stress levels were applied (%70 irrigation and %30 irrigation). Each treatment, including the control, was conducted with four replications.

The pots used in the research were filled with a pre-prepared soil mixture in equal amounts, and to determine the water-holding capacity of the pots, they were initially saturated with water. The pots were left to drain under gravity for 24 hours, and their water-holding capacities were determined by weighing. After saturating the pots, seedlings were planted. Following the adaptation of the seedlings to the pots (10 days after planting), water stress applications began. The control pots were weighed every 3 days to determine the amount of evaporation and water used by the plant in terms of weight, and the amount of water lost was replenished with irrigation water. The reduced water amounts were applied through the use of a measuring cup for irrigation purposes. The water stress treatments of 70% irrigation and 30% irrigation were determined based on the amount of irrigation water applied to the control pots in each irrigation. Çanakkale Municipality tap water was used as irrigation water. The quality of the irrigation water was determined as T2A1.

At the end of the cultivation period, the yield per plant (g per plant), yield per decare (kg per da), fruit length and diameter (mm) were determined by measuring the harvested fruits.

For the determination of internal proline content, pepper leaves were frozen in liquid nitrogen, and 0.5 g samples were weighed and homogenized in 10 ml of 3% 5-Sulfosalicylic acid using a homogenizer for 2 minutes at maximum speed at -18°C until analysis. Then, the homogenate was filtered through Whatman No. 2 filter paper and transferred to tubes. Two millilitres of the filtrate were mixed in a sealed test tube with 2 ml ninhydrin and 2 ml glacial acetic acid, and the reaction was carried out at 100°C for 1 hour in a water bath set at 100°C. The tubes were then placed in an ice bath to complete the reaction. 4 ml of toluene were added, and the reaction mixture was mixed for 15-20 seconds using a tube mixer. The chromophore-containing phase was carefully aspirated with a fine-tipped pipette and transferred to spectrophotometer tubes. Absorbance readings at 520 nanometers were taken when the spectrophotometer tubes reached room temperature. Toluene was used as a control (Bates et al., 1973).

The Folin-Ciocalteau method, as described by Singleton et al. (1999) was used to determine the total phenolic content ($mg^{-1}g$) in peppers. Initially, 100 µl of pepper extract was vortexed with 900 µl of distilled water, 5 ml of 0.2 N Folin-Ciocalteau reagent, and 4 ml of 7.5% sodium carbonate solution in test tubes. The resulting solution was allowed to stand at room temperature for 2 hours, and then absorbance values at 765 nanometers were measured using a spectrophotometer. The total phenol content of the pepper samples was expressed as "mg of gallic acid equivalents per 100g of fresh weight" (mg GA^{-1}100g FW).

The total flavonoid concentrations ($mg^{-1}g$) of pepper extracts were measured using aluminium-based colorimetric analysis (Shraim et al., 2021). Test tubes were sequentially filled with 100 µl of pepper extract, 100 µl of 1 M potassium acetate, 100 µl of 10% aluminium nitrate, and 4.4 ml of 96% ethanol. After incubating the samples in the dark at room temperature for 40 minutes, absorbance values at 415 nanometers were measured using a spectrophotometer. The total flavonoid content of the pepper samples was calculated as "mg of quercetin equivalents per 100g of fresh weight" (mg $quercetin^{-1}$100g FW).

The CUPRAC method, which is a copper-reducing antioxidant capacity test according to Apak et al. (2004), is based on an electron transfer method to determine the ability of plant samples to reduce copper ions (Cu^{+2}). As described by Marangoz (2016) 30 µL of plant extracts were mixed with 1 ml of 0.01 M copper(II) chloride, 1 ml of 7.5 x 10-3 neocuproine, 1 ml of 1 M pH 7 ammonium acetate, and 1080 µL of distilled water. After incubation at 20°C for 30 minutes, absorbance values at 450 nm were determined using a spectrophotometer. Antioxidant

activity was calculated as trolox equivalents in mg per 100g of fresh sample (mg trolox $^{-1}$100g FW) using a standard calibration curve.

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method as described by Brand-Williams et al. (1995) and Ak and Türker (2018). Different concentrations of methanol-diluted pepper extracts were mixed with DPPH solution after being allowed to stand at room temperature for 30 minutes.

The absorbance values of the prepared samples were measured at 515 nanometers using a UV spectrophotometer. The percentage of DPPH radical scavenging activity was determined using the following equation:

$$DPPH\,(\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100"$$

(Note: In the equation, $A_{control}$ represents the absorbance of the control sample, and A_{sample} represents the absorbance of the sample.)

The IC_{50} inhibition values were calculated considering the concentration at which 50% of the total DPPH radicals were captured, with lower IC_{50} values indicating higher radical scavenging activity. In this test, butylated hydroxytoluene (BHT) was used as a positive control.

Analysis of variance (ANOVA) was used to determine the level of differences among the data obtained from the experimental subjects, and the Duncan test was applied to classify these identified differences.

Results and Discussion

The total irrigation water applied to the Hungarian hot pepper variety, which is the subject of the research, and some morphological measurement results obtained after harvest are given in Table 1. Irrigation water was applied at 3-day intervals until the end of the research, and the total water consumption for the control plants was determined to be 518.2 mm. The average yields per plant decreased with water stress by the end of the research. The highest yield was determined as 269.42 g from the trial with full irrigation water application, while the lowest yield was measured as 93.33 g from the trial with 30% irrigation water application. In the study conducted by Demirel et al. (2012), it was found that along with water scarcity, the yield and quality parameters of pepper plants decreased. They have stated that to obtain high yield and quality products, it is essential to provide the required amount of water in a timely manner and that there will be decreases in yield in case of water scarcity.

When the yields per decare were calculated, they were determined as 1122.40 kg^{-1}da for the 100% irrigation trial, 625.49 kg^{-1}da for the 70% irrigation trial, and 388.79 kg^{-1}da for the trial with 30% irrigation water application. According to these results, it can be understood from the yield results that the pickling hot pepper plant is sensitive to water. In other words, unless necessary, the application of water restriction in pickling hot pepper cultivation is not recommended according to the research results.

When looking at the measurement values of single fruit weight, fruit count, fruit diameter, and length, statistically significant differences were detected as observed in the yield values. It was determined in the study that as water stress increased, the parameters determining the quality of pickling hot peppers also decreased. The highest fruit count was determined as an average of 85 fruits from the fully irrigated trial, while the lowest fruit count was found to be 26 fruits from the trial with severe water stress, which received 30% irrigation.

At the end of the research, the analysis of some biochemical components was conducted on samples taken from each trial, and the results obtained are presented in Table 2.

The amounts of phenolic compounds varied between 0.827-1.536 mg^{-1}g depending on the applied irrigation water levels. Phenolic compound accumulation increased with the increasing water stress. The highest accumulation of phenolic compounds occurred in the trial with 30% irrigation water application. Phenolic compounds have received significant attention as a result of in vitro and in vivo studies due to their effective antioxidant capacity. They can be divided into three groups: free, esterified, and insoluble bound forms. These are determined based on whether they exist in a free form or are covalently bound to other molecules like fatty acids (soluble esters) or macromolecules (insoluble bound phenolics). Most insoluble bound phenolic compounds form covalent bonds with cell wall components such as pectin, cellulose, arabinoxylans, and structural proteins and are relatively more abundant in foods compared to soluble phenolic compounds (20-60% in vegetables, fruits, and legume seeds) (Nayak et al., 2015; Shahidi and Yeo, 2016). Considering the analytical methods available to measure the contents of insoluble bound phenolics in natural sources, it can be assumed that the amount of insoluble bound phenolics may be higher than expected (Shahidi and Yeo, 2016).

Table 1. Irrigation water amount and some morphological measurement results.

Treatments	IWA	YP	YD	SFW	FC	FD	FL
100% irrigation	518.2	269.42a	1122.40a	2.37a	85a	49.66a	76.82a
70% irrigation	362.7	150.14b	625.49b	1.49b	62b	39.87b	59.45b
30% irrigation	155.5	93.33c	388.79c	0.90c	26c	32.15c	21.38c

IWA: Irrigation Water Amount (mm); YP: Yield (g^{-1} plant); YD: Yield (kg^{-1} decare); Single Fruit Weight (g); FC: Fruit Count (number); FD: Fruit Diameter (mm); FL: Fruit Length (mm)

Table 2. Applied irrigation water amounts and some biochemical parameter results.

Treatments	IWA	PC	TF	CUPRAC (mg^{-1}g)	DPPH (mg^{-1}g)	Proline (µg^{-1}g)
100% irrigation	518.2	0.827^{c}	0.146^{b}	0.717^{c}	0.763^{a}	308
70% irrigation	362.7	1.170^{b}	0.373^{ab}	1.541^{b}	0.630^{b}	482
30% irrigation	155.5	1.536^{a}	0.412^{a}	1.882^{a}	0.568^{c}	641

IWA: Irrigation Water Amount (mm); PC: Phenolic Compound (mg^{-1}g); TF: Total Flavonoids (mg^{-1}g)

The total flavonoid content, which is among the biochemical components, has been synthesized to a greater extent with increasing water stress, similar to the observed increase in phenolic compound levels. The highest amount, 0.412 $mg^{-1}g$, was measured in the trial with 30% irrigation water application, while the lowest amount was measured in the peppers harvested from the control plots with 100% irrigation water application.

Plants develop resistance strategies to adverse conditions such as osmotic adjustment, involving components like potassium, proline, glycine betaine, and soluble sugars (Turan et al., 2009; Benhassaini et al., 2012). They stimulate antioxidant enzyme activities by increasing some plant growth regulators, leading to an accumulation of antioxidant substances (Çelik and Atak, 2012). This phenomenon was also observed in pickling hot peppers when looking at the results of our study. Antioxidant contents were determined using two different methods. According to the analysis results conducted using the CUPRAC method, the lowest antioxidant content was determined as 0.717 $mg^{-1}g$ in the peppers harvested from the control group. The highest antioxidant content was determined as 1.882 $mg^{-1}g$ in the peppers treated with 30% irrigation water. The results of the antioxidant substance analysis using the DPPH method were similar to those obtained with the other method. In their study using different pepper varieties, Uğur and Saka (2022) have determined that the antioxidant content of hot peppers is higher than that of other varieties. Furthermore, in the results of the study conducted using only pickling hot peppers, it was found that the antioxidant levels increased in conjunction with water stress.

When plants encounter any stress during their growth period, they accumulate a range of secondary metabolites in their bodies, especially amino acids (Giordano et al., 2021). Among these substances, proline is synthesized as an amino acid that plays a beneficial role when plants are exposed to stress conditions (Hayat et al., 2012). According to the proline analysis results obtained at the end of the study (Table 2), proline in the leaves varied between 308 and 641 $\mu g^{-1}g$. Based on these findings, it was determined that as soil moisture content decreased, the amount of proline in the leaves increased to enhance the plant's resistance to these conditions, and it was observed that it was synthesized more in response to water restriction. Escalante-Magaña et al. (2019) stated that proline concentration significantly increased with increasing water stress in pepper species (*Capsicum* sp.) compared to control plants. The best characteristic of proline is its strong osmotic protective property. Additionally, previous research has identified significant increases in proline levels under conditions such as salt and drought stress (Mafakheri et al., 2010; Erken et al., 2013; Özkoku et al., 2019).

Conclusion

In today's world, both the increasing population growth and global warming have made water resources even more critical in terms of meeting food needs worldwide. When we look at the scientific studies examining the relationship between water and yield in plant production, it can be observed that these studies aim to determine the changes in the biochemical properties and economic yield values of plants grown at different water levels. The changes in the chemical substances synthesized by plants under different water levels are crucial in terms of their effects on human health.

When the research results are evaluated together, it is determined that hot pepper plants for pickling are sensitive to water. Water shortage should be avoided during the cultivation period unless absolutely necessary. In regions with high temperatures and limited water resources, insufficient irrigation conditions can lead to significant yield losses in the cultivation of hot peppers for pickling. If water shortage is necessary, it has been found that applying the minimum amount of water restriction, preferably as little as possible, is important in terms of yield and quality parameters.

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Modeling of The Nitrogen Requirement of Winter Wheat for Protein Content Using Optical Sensor in Central Anatolia Region of Türkiye

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ABSTRACT

Chlorophyll meters manage both the amount and duration of nitrogen fertilizer application based on the principle that the chlorophyll or nitrogen content of plants should be maintained throughout their development. For smallholders in developing countries, the use of a hand-held meter to manage nitrogen fertilizer in rice and wheat is the most popular method. The adoption of nitrogen management strategies based on close sensing using chlorophyll meters and optical sensors will largely depend on the inclusion of specific economic analysis in future research. The importance of using sensors and chlorophyll meters for nitrogen fertilizer management depends on how successful current practices have been. In this study, five different nitrogen rates (0, 80, 120, 160, 200 kg N ha-1) were applied to two different wheat varieties, and the effect of these different nitrogen rates on wheat protein content was investigated in a randomized block design. A quadratic polynomial model described the relationship between protein content and nitrogen rates.

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Introduction

Nowadays, fertilizer applications for quality and yield increase have recently started. This research aims not to apply fertilizers directly for quality increase but to determine the optimum nitrogen rate used in variable rate fertilizer applications by using optical sensors to reach the maximum protein amount. With the help of the equations obtained as a result of the research, it is aimed to maximize the quality and minimize the nitrogen use as much as possible by determining the most appropriate nitrogen dose for the area. Therefore, the optimum fertilizer dose was determined by applying variable rate nitrogen, an essential part of precision agriculture practices.

As a result of high levels of nitrogen fertilizers, the amount of nitrate that is washed from the soil and mixed with drinking water and rivers increases. Some harmful substances are produced in plants growing in soils with excess nitrogen fertilizer application. As a result of intensive chemical fertilization, the soil becomes poorer in organic matter, leading to decreased biological activities and deterioration of the soil structure. If this continues, soils will deteriorate yearly, plant growth will slow down and stop depending on the intensity of chemical fertilization, and yield will decrease.

As a result of intensive chemical fertilization, the amount of organic matter in the soil's humus ratio will decrease, biological activity will decrease, and the fertilizers given will be washed away because they cannot hold in the soil. The transformation of plant nutrients into the form that plants can take will stop, and thus, the physical and chemical properties of the soil will deteriorate. As a result, the upper parts of the soil will become sandy, and the lower parts will become stony.

One of the most significant factors causing environmental pollution and the deterioration of the natural balance is the intensive use of chemicals in agricultural activities. Moreover, the agricultural method using chemicals causes ecological pollution and disruption of the natural balance and threatens the lives of all living things by reaching them through the food chain. This threat's continuity and rapid progression make it more and more challenging to return to nature.

The part of inorganic fertilizers containing nitrogen and phosphorus compounds that plants do not take up is washed out of the soil by rain and irrigation water and transported to the water environment. Nitrogen and phosphorus are converted into nitrate and phosphate due to biochemical reactions. These reduce the water-holding capacity of the soil. Nitrate in chemical fertilizers mixes with soil water as rainwater seeps through the soil. When nitrate concentration in water taken from wells and used as drinking water exceeds 45 mg L^{-1}, it becomes toxic, and methemoglobinemia occurs in infants. Oxygen consumption and fish deaths occur in lakes and rivers. In addition, it causes low crop potential, quality deterioration in crop production, corrosion of soil fertility, deterioration of soil reaction, and deterioration of the elemental balance in the soil.

As a result of this research, reducing all these adverse side effects will be possible. In addition, one of the critical issues is the economic losses caused to farmers by unnecessary fertilizer use. Considering that the price of 1 ton of DAP (diammonium phosphate) fertilizer is 1500 USD today, it is evident that the farmer will gain if the extra 1 kg of fertilizer to be applied per decare is saved. In our country, there is currently no practical tool for farmers to use this method. The aim is to determine the optimum amount of nitrogen to be applied as a result of the research to be carried out with the help of sensors and to maximize the protein ratio in wheat with the least input.

Materials and Methods

The research was conducted in the experimental fields of Ankara University Faculty of Agriculture Haymana Research and Application Farm (39º37'00.05" N - 32º41'39.09" E) with two different red hard bread wheat varieties under irrigated conditions for two years. The experiment was carried out in a factorial experimental design with four replications in randomized experimental blocks consisting of 2 factors: nitrogen level and wheat varieties. 5 different nitrogen levels were used as 0, 80, 120, 160, and 200 kg N ha^{-1}. One of the varieties used in the experiment was Bezostoya1, and the other was Ahmetağa. Sowing was carried out on the 21st of October in the first year and on the 22nd of October in the second year, known to be the most suitable time for winter wheat sowing in the Central Anatolia Region. After the preliminary studies and soil preparation necessary for sowing, the sowing process was carried out as 5N (nitrogen levels) x 2Ç (wheat seed) × 4T (number of replications) = 40 plots.

Diammonium phosphate (DAP; 18% nitrogen and 46% phosphorus) was used as base fertilizer in Central Anatolia. Therefore, DAP fertilizer was used at a dose of 200 kg ha^{-1} in farmer applications. In the experiment, Ammonium Nitrate (AN; 33% nitrogen) was used as a nitrogen source in top dressing, and (G1;0), (G2;80), (G3;120), (G4;160), (G5;200) kg N ha-1 doses were used. After harvesting, the products obtained from each plot were weighed, and yield values were obtained and sent to the laboratory for protein analysis.

NDVI, Normalized Difference Vegetation Index, has an algorithm based on the principle that a healthy plant absorbs visible light and reflects most of the near-infrared light. In contrast, an unhealthy plant reflects more visible and less near-infrared light. NDVI readings were taken with a GreenSeeker sensor (NTech Industries, INC., USA). Red light is absorbed by the plant during photosynthesis as an energy source. A healthy plant absorbs more red light, while NIR light is largely reflected. The light reflected by the plant is measured by a photodiode located at the front of the sensor. The values obtained vary between -1 and +1. Where healthy and dense vegetation is present, the index value approaches +1, whereas where unhealthy and weak vegetation is present, the index value approaches -1.

The normalized vegetation index is formulated as follows.

$$NDVI = \frac{NIR-R}{NIR+R}$$

Normalized vegetation index values, or the ratio of the near-infrared band to the red band, provide information on green vegetation cover and the areas that are poorly vegetated or empty without vegetation. Moreover, the closer the vegetation index is to a value of 1, the denser the vegetation, and the closer to zero, the vegetation cover decreases. When it is negative, the areas are devoid of vegetation.

Flavonol and chlorophyll-to-flavonol ratio readings (Nitrogen Balance Index, NBI) were performed with the Force-A Dualex Scientific Spad Meter optical sensor. Flavonoids are polyphenolic compounds with different skeletal structures found in various plants; these differences in their skeletal structures include flavone, flavonol, flavonone, etc. They take names. The type of flavonoid found in wheat is flavonol. As a result of the research, it was found that the chlorophyll-flavonol content ratio (NBI) can be used in protein estimation. By measuring the NBI value in the plant with the help of this sensor, raw data that can be used to estimate the protein content are revealed.

If nitrogen fertilization is appropriately done, the plant produces chlorophyll, and due to nitrogen deficiency, the plant has flavonol. Flavonoids are found in the epidermis layer of the plant and absorb ultraviolet wavelength rays. The sensor measures the near-infrared rays emitted by the chlorophyll layer from both sides of the leaf. Measurements are carried out with the readings obtained from the leaf during the spike period, called NBI.

Sowing was done in 8 rows with 15 cm row spacing using a single row manual seeder. A plot length of 6 m was used, and 50 cm was left between the blocks.

NDVI and NBI readings

According to the Zadoks convention (Zadoks et al., 1974), NDVI readings were taken with the GreenSeeker device at Z21, Z30, Z37, Z50, Z55, and Z60, and these values were used to calculate RI (Response Index) and INSEY (In Season Yield Estimation) values. RI and INSEY equations were used in the evaluation of NDVI readings. Accordingly, the $RI_{(NDVI)}$ values obtained by dividing the NDVI value of the plots giving the maximum NDVI value by the NDVI values of the unfertilized control plots were compared with the $RI_{(HARVEST)}$ value obtained by dividing the maximum yield obtained at harvest by the yield obtained from the control plots as a result of the correlation analysis the $RI_{(HARVEST)}$ value and the $RI_{(NDVI)}$ value gave the highest R^2 value from which period readings were obtained. Readings were taken in that period in the subsequent studies for recommendation. Force-A Dualex

Scientific Spad Meter sensor was used to take ten readings from each plot during the wheat spike period. When both readings were evaluated statistically, a very close relationship was found. Therefore, these NDVI values were used as the average of the NDVI readings obtained with the GreenSeeker and Force-A Spad Meter to estimate protein and to find the optimum point in the yield-protein relationship. Image 1 and Image 2 show pictures of different reading periods.

Evaluation of The Data

Analysis of variance was used in the statistical evaluation of the data obtained from the research, and the Tukey multiple comparison test was applied to determine the difference between groups. The SAS package program was used for this purpose. The analysis results were evaluated regarding different nitrogen rates and seed varieties. Image 3 shows the harvesting process and samples obtained from each plot.

A quadratic polynomial model was used to predict yield and protein versus applied nitrogen rate:

$$f(N) = c + b.N + a.N^2$$

$$g(N) = \beta + \alpha.\mathrm{N}$$

Where;
f(N) : Yield (tons/ha),
g(y) : Protein content (%),
N : Fertilizer rate and
A, b, c: regression coefficients of yield and protein response functions to nitrogen.

The models obtained for yield and protein were used for the economic optimum nitrogen rate. Determination of the optimum nitrogen rate in nitrogen fertilizer consumption is vital in wheat production due to its effect on quality and yield. The average yield and protein results obtained were used to determine this rate.

RI (Response Index) was used to determine the optimum fertilization time. This index was calculated for NDVI and yield values. Accordingly, RINDVI values obtained by dividing the NDVI value of the plot's NDVI value by the NDVI values of the other plots were compared with the RIharvest value obtained by dividing the maximum yield obtained at harvest by the yield obtained from the different plots. As a result of the correlation analysis, the RINDVI value giving the highest R^2 value with the RIharvest value was obtained from which period readings were obtained. Then, readings will be taken in that period in the studies for variable rate nitrogen application. In the experiment, RINDVI and RIhasat are calculated using the following equations Mullen et al. (2003):

$$RI(NDVI) = \frac{\mathrm{NDVI(max)}}{\mathrm{NDVI(control)}}$$

$$RI(harvest) = \frac{\mathrm{Yield(max)}}{\mathrm{Yield(control)}}$$

After determining the optimal fertilization time, the relationship between NDVI values and fertilizer rates for that period was defined, and the nitrogen status of the plant was determined by using the regression equation obtained by the linear regression method. INSEY values were used to estimate potential yield with the GreenSeeker sensor (Franzen et al., 2013). INSEY (Season Yield Estimation) is the value obtained by dividing the NDVI reading in any period by the cumulative growing degree days (CGDD) from planting to the reading date (Lukina et al. 2001; Teal et al. 2006).

a b c d e f

Image 1. 1st year, 1st reading period (a), 2nd year, 1st reading period (b), 1st year, 2nd reading period (c), 2nd year, 2nd reading period (d), 1st year, 3rd reading period (e), 2nd year, 3rd reading period (f)

a b c d e f

Image 2. 1st year, 4th reading period (a), 2nd year, 4th reading period (b), 1st year, 5th reading period (c), 2nd year, 5th reading period (d), 1st year, 6th reading period (e), 2nd year, 6th reading period (f)

Growing-degree days (GDD) integrate excess temperatures on days with temperatures bounded by maximum and minimum values. GDD is defined by the following equation:

$$GDD = (T_{max}+T_{min})/2-T_{basic}$$

GDD : Growth-degree-days,
T_{max} : Daily maximum air temperature,
T_{min} : Daily minimum air temperature and
T_{basic} : The basic threshold temperature at which crop development begins.

The equation obtained as a result of regression analysis in which INSEY values are taken as independent variable (x) and wheat yields obtained from the same plots as dependent variable (y) can be used as a calibration equation in variable rate fertilizer application.

Image 3. Harvesting process and products obtained from each plot

Table 1 Effect of different fertilizer applications on protein at the experimental location

1st year	
Fertilizer Dose	Protein (P=0.006)
1	15.280±1.273 B
2	15.747±1.381 AB
3	16.717±1.086 B
4	17.712±1.132 B
5	18.018±1.805 B
2nd year	
Fertilizer Dose	Protein (P=0.025)
1	15.530±0.524 B
2	15.01±2.82 AB
3	17.015±0.925 A
4	17.713±1.921 A
5	17.517±0.658 A

*Means marked with the same letters are not different from each other (P<0.05).

Table 2. Yield and protein response functions and regression curves and R2 values corresponding to nitrogen in wheat

Seed variety	Protein response function		
	α	β	R^2
Bezostaya	0.6765	14.105	0.6746
Ahmetağa	0.935	14.427	0.5684
Ave.	0.8057	14.176	0.535

Results and Discussion

Laboratory Results and Statistical Analysis

The analysis of variance showed that the difference between the parameters was statistically significant in terms of different fertilizer rates. The results obtained from the analysis are given in Table 1.

The experiment obtained $p<0.05$ statistically significant results between fertilization levels and protein values. p values were determined as 0.006 and 0.025 for the 1st and 2nd years for protein in terms of fertilization levels, respectively. In addition, according to the results obtained, the effect of seed varieties used in the experiment on protein was statistically significant. Based on the results obtained at the experimental location, the 5th fertilizer dose (20 kg N da^{-1}) in the 1st year and the 4th fertilizer dose (16 kg N da^{-1}) in the second year were determined as the appropriate fertilizer rate for the highest protein content.

Determination of Optimum Fertilizer Rate

Determining the optimum nitrogen rate in nitrogen fertilizer consumption is important because of its effect on quality and yield in wheat production. On the other hand, as nitrogen is a mobile element, its unnecessary use causes groundwater pollution. Therefore, it is necessary to determine the optimum and economical fertilizer rate. The average of the protein results obtained was used to determine this rate. The results are shown in Table 1. Table 2 shows the protein response functions corresponding to nitrogen in wheat, regression curves, and R^2 values. The protein response functions corresponding to nitrogen in wheat are also given in Image 4.

Quadratic polynomial and linear models were used to predict yield and protein against applied nitrogen rate, respectively (Cerrato and Blackmer 1990);

$$f(N) = c + b.N + a.N^2$$

$$g(N) = \beta + \alpha.N$$

f(N) : Yield (tons/ha),
g(y) : Protein content (%),
N : Fertilizer rate and
a, b, c: regression coefficients of protein response functions to nitrogen.

The obtained models were used for the economic optimum nitrogen rate. Determination of the optimum nitrogen rate in nitrogen fertilizer consumption is essential in wheat production due to its effect on quality and yield. The average yield and protein results obtained were used to determine this rate. The additional price given according to the protein ratio in wheat with less than 1% of wheat fly damage is defined as follows.

Protein 12-12.5% to 1
Protein between 12.5-13% 2.5
Protein between 13-13.5% 3
Protein 13.5-14.5% to 4.5%
6% protein between 14-14.5%
7% on 14.5% protein.

Table 3. Relationships between RINDVI and RIHarvest obtained after fertilization.

RI_{NDVI}	RI_{hasat} (R-sq%)
1.RI_{NDVI}	8.82
2.RI_{NDVI}	11.22
3.RI_{NDVI}	69.38
4.RI_{NDVI}	77.52
5.RI_{NDVI}	76.58
6.RI_{NDVI}	65.31

Table 4. Effect of different fertilization rates on post-fertilization NDVI readings

Fertiliser doze	1st NDVI values	2nd NDVI values	3rd NDVI values	4th NDVI values	5th NDVI values	6th NDVI values
0 kg N ha^{-1}	0.40±0.08 A	0.61±0.09 A	0.68±0.067 B	0.62±0.04 B	0.65±0.10 AB	0.57±0.11 A
80 kg N ha^{-1}	0.46±0.15 A	0.64±0.09 A	0.68±0.04 B	0.70±0.07 AB	0.61±0.04 B	0.55±0.09 A
120 kg N ha^{-1}	0.50±0.10 A	0.65±0.06 A	0.73±0.07 AB	0.72±0.07 A	0.70±0.06 AB	0.49±0.11 A
160 kg N ha^{-1}	0.47±0.18 A	0.71±0.06 A	0.78±0.03 A	0.75±0.02 A	0.71±0.09 AB	0.47±0.06 A
200 kg N ha^{-1}	0.43±0.19 A	0.69±0.04 A	0.75±0.05 AB	0.77±0.02 A	0.74±0.05 A	0.58±0.10 A

* Means marked with the same letters are not different from each other (P<0.05)

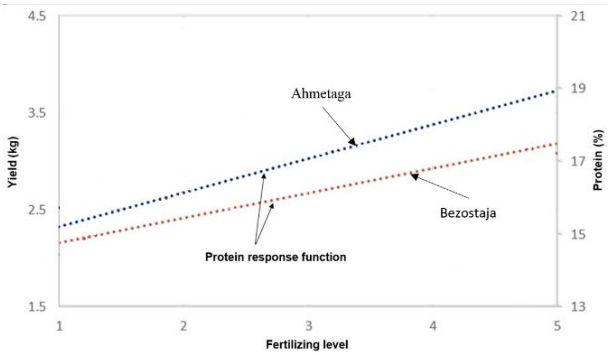

Image 4. Protein response functions corresponding to nitrogen in wheat

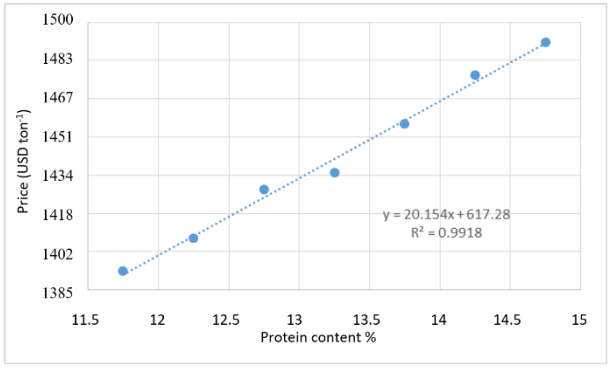

Image 5. Protein content of wheat and price per ton (TL)

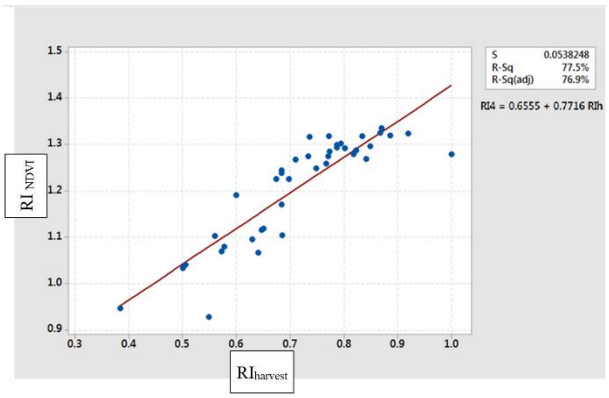

Image 6. Regression curves and equations between RINDVI and RIHarvest obtained from the 4th reading after fertilization

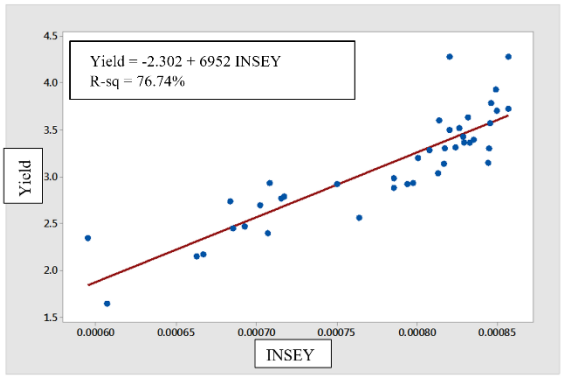

Image 7. Relationship between INSEY and yield values

Since the base price is set at 1500 USD/ton for wheat, the following relationship can be written between price and protein content in the range of 12-15% protein content:

$$F_b(\text{Price}) = 20.154\,\text{Protein}\% + 617.28$$

Image 5 shows protein content of wheat and price per ton (TL). Using equations, we can calculate the net income from wheat production by considering the amount of nitrogen fertilizer used as input (þN=1.74 TL/kg):

$$NR = F_b.f(N) - p_N.N$$

For Bezostaya: $NR = -1.73N^3 - 100.58N^2 + 934.89N + 1036.1$

For Ahmetağa: $NR = -1.64N^3 - 9.66N^2 + 272.6.89N + 511.7$

According to the method proposed by (Cerrato and Blackmer 1990, the optimum nitrogen rate is calculated by setting the first-order derivative of NR equal to zero.

$$\frac{\partial(NR)}{\partial(N)} = 0$$

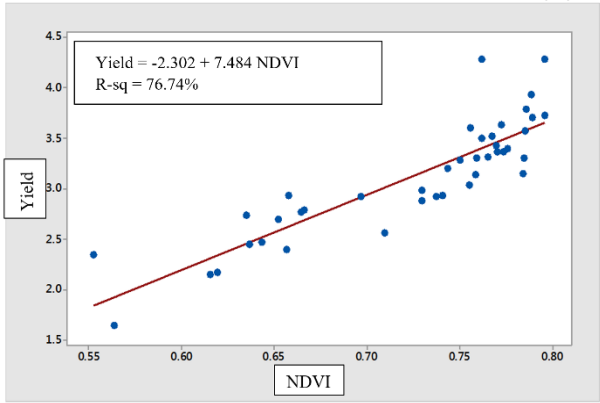

Image 8. Relationship between yield and NDVI values

As a result of this equation, the optimum fertilizer rate was determined as 16.76 and 20.6 kg da^{-1} for Bezostaya and Ahmetağa varieties, respectively.

NDVI and yield values at harvest were used to determine the optimum fertilization time. Response indexes were calculated using NDVI and yield values from different periods. The results of linear regression to determine the relationship between the calculated RINDVI and RIharvest values are given in Table 3. The average of the values obtained from the two locations was used to construct these indices.

As shown in Table 3, as a result of the regression analysis, the R^2 value was obtained in the 4th reading period after fertilization (CGDD=928.9). Therefore, the 4th reading period after fertilization was determined as the most suitable period for the variable rate application regarding NDVI variability. Image 6 shows that regression curves and equations between R_{INDVI} and $RI_{Harvest}$ were obtained from the 4th reading after fertilization.

When the NDVI values obtained were analyzed, NDVI values differed between the plots in some reading periods in both experimental locations. As a result of the analysis of variance, the difference between the plots was found to be statistically significant in Year 1, 3rd, 4th, and 5th readings and in Year 2, 3rd and 4th readings (Table 4).

INSEY values were used to estimate potential yield with the Green Seeker sensor. To create this equation, INSEY values calculated from NDVI readings (with maximum correlation value with RIhasat) obtained from the plots at the 4th reading after fertilization (928.9 CGDD) were used. Image 7 shows relationship between INSEY and yield values. The resulting calibration equation was as follows during the planned period:

Yield = -2.302 + 6952 INSEY R-sq 0 76.74%

Since the CGDD for the period is 928.9 °C and INSEY=NDVI/CGDD, the relationship between yield and NDVI can be calculated with the following equation:

Yield = 7.484 NDVI - 2.302

Image 8 shows that relationship between yield and NDVI values.

The yield obtained with the help of this equation determines the potential wheat yield without using nitrogen fertilizer. To create a calibration equation based on NDVI used in variable rate nitrogen applications, NDVI values obtained from the 4th readings, where the difference was statistically significant and determined at fertilization, were used.

Worldwide, protein deficiency is a significant problem in human nutrition, second only to calorie deficiency (Robinson et al. 1986). In developing countries, 90% of the average diet comprises cereal-based foods (Graham and Welch 1996).

The importance of wheat protein in nutrition and the necessity for Turkey, which is hoped to become a net exporter of wheat and wheat products if it can improve its production to meet and exceed international quality standards, make protein a top priority.

Nitrogen Use Efficiency (NUE) was divided into uptake and utilization efficiency as early as the 1980s (Moll et al. 1982), and this distinction facilitated investigations. Dhugga and Waines (1989) stated that uptake efficiency is more critical than utilization efficiency, especially when nitrogen content is high. Similarly, Van Ginkel et al. (2001) stated that uptake efficiency is more important under conditions with high nitrogen content. In contrast, varieties with higher utilization efficiency may be preferred under low-input farming conditions where nitrogen content is insufficient.

Protein contents in wheat grain are determined not only by genotypic but also by environmental sources of variation, which makes it necessary to test the ability of cultivars selected for high protein contents to maintain these traits under different environmental conditions, as well as the significant environment x genotype interaction, which makes it difficult to use molecular marker techniques reliably in large breeding programs (Pena, 2002).

In most cases, grain protein concentration increases with increasing temperature and decreasing rainfall. A study conducted in the Western Transition Zone in Turkey observed that rainfall during the active growth period negatively correlated with grain protein content of all cultivars (Kalaycı et al. 1996). It is stated that drought and high-temperature decrease carbohydrate accumulation and relatively increase nitrogen accumulation (Panozzo and Eagles, 1999).

It is stated that bread quality is highly affected by climatic conditions, especially during grain filling, and if cool and humid weather prevails during this period, bread quality decreases (Johansson and Svensson 1998, 1999; Johansson et al. 2002). Among the literature findings, temperatures above 20 °C during grain filling provide high protein concentration (Altenbach et al. 2003, Gooding et al. 2003), and weather conditions affect protein polymerization and baking quality (Johansson et al. 2003).

Many researchers have reported the effect of soil inorganic nitrogen content on grain protein. In the trials conducted in Eskisehir and the Western Gateway Region, soil nitrogen was essential for protein. Grains containing more than 13% protein were obtained only when the total inorganic nitrogen (nitrate + ammonium) content at 120 cm depth of the soil profile exceeded 10-11 kg ha^{-1}, and slightly more nitrogen was used than required for optimal yield. In fields where soil inorganic nitrogen was below 10 kg/ha, no protein above 11% could be obtained even at the highest dose (12 kg N ha^{-1}) (Kalaycı et al. 1996).

Regarding the effect of soil nitrogen on plant nitrogen uptake, it has been reported that the available nitrogen in the soil is vital for nitrogen uptake (Ortiz-Monasterio et al. 1997) and that there is a high correlation between soil nitrate content and nitrogen uptake of barley.

Smil (1997) stated that it is only possible to reduce the use of nitrogen fertilizers by finding more suitable fertilization methods and increasing their efficiency. However, it is noted that the uptake rate of applied nitrogen by wheat under dry farming conditions is below 50%, mainly due to evaporation losses from surface applied nitrogen fertilizer (Fillery and McInnes 1992). It has been reported that volatilization losses in the form of ammonia can exceed 40%, especially when urea fertilizer is applied to the surface without mixing with the soil in this way (Flower and Brydon, 1989) and that these losses are generally more significant in the presence of high temperature, high pH and stubble residues on the surface (Raun and Johnson, 1999).

It has been reported that there is a relationship between leaf nitrogen concentrations and maximum photosynthesis values, i.e., some nitrogen is present in the leaves even when photosynthesis is zero, possibly indicating non-photosynthetic nitrogen accumulation, but that this is more limited in C3 plants, which is evident in C4 species, possibly as a result of selection pressures for yield (Zhang et al., 2020). In this case, it seems to be the best way to carry out the calibration studies mentioned above separately for yield and protein.

In the evaluation of NDVI readings, the concepts of RI (Response Index) (Mullen et al. 2003) and INSEY (In Season Yield Estimation) (Raun et al. 2002) are utilized. Accordingly, in calibration studies, $RI_{(NDVI)}$ values obtained by dividing the NDVI value of the plots giving the maximum NDVI value by the NDVI values of the unfertilized control plots are compared with the $RI_{(HARVEST)}$ value obtained by dividing the maximum yield obtained at harvest by the yield obtained from the control plots (Johnson et al. 2002) and as a result of the correlation analysis, the $RI_{(NDVI)}$ value that gives the highest R^2 value with the $RI_{(HARVEST)}$ value is compared with the $RI_{(NDVI)}$ value obtained from which period readings are received. Then, readings are taken in that period for recommendation studies to be carried out in farmers' fields.

In the study conducted in Oklahoma, according to the Feekes convention (Large, 1954), readings obtained at 5 (beginning of emergence), 9 (end of emergence), and 10.5 (flowering) were similarly effective (Mullen et al. 2003). Still, in large farmer field applications, recommendations are guided by readings taken at Feekes 5, the beginning of emergence, which is equally effective as the others. The INSEY value is calculated by dividing the NDVI value by the number of days from planting to the day of the reading when the average temperature was above 4.4 °C (Raun et al. 2002). Then, the equations obtained as a result of regression analysis, where the INSEY values calculated from the NDVI readings of the plots are taken as the independent variable (x) and the grain yields obtained from the same plots as the dependent variable (y), are used as calibration equations that guide the practices in farmers' fields.

The method used in this study increases wheat yield and protein content. This method allows more productive areas in the field to be fertilized at the optimum rate and reduces the nitrogen cost. This study found the effects of different nitrogen rates on yield and quality parameters statistically significant.

As a result of the analysis, the relationship between seed variety and yield, the relationship between fertilizer dose and yield, and the relationship between seed variety and fertilizer interactions were found to be statistically significant. Similarly, the relationship between seed variety and protein ratio and between seed variety and fertilizer interaction in terms of protein ratio were statistically insignificant. However, the relationship between fertilizer dose and protein, essential for this study, was statistically significant.

Determination of economic optimum fertilizer rates has been done using yield and protein values for wheat. This method has been used for in-season nitrogen estimation in wheat (Raun et al. 1999, Solie et al. 1996).

The aim of this study, in summary, is to investigate what should be done to improve grain protein coverage, which is one of the most critical problems in wheat production and marketing in Turkey, and to determine the most appropriate nitrogen fertilization management system and nitrogen fertilizer application method that will minimize nitrogen losses and maximize nitrogen utilization efficiency of the plant in terms of nitrogen nutrition, which is one of the most critical factors determining wheat yield and quality, especially protein coverage, this study aims to contribute to both input and production economy by developing and disseminating a system that will regulate nitrogen fertilizer applications, which are generally made based on average recommendation values, according to the course of the year, so that wheat grain yield and quality will be at the highest level.

By determining the most appropriate nitrogen fertilizer application method in which nitrogen losses will be at the lowest level and the efficiency of nitrogen use of the plant will be at the highest level and reflecting it to practice, both the national economy and the input economy for the farmer will be contributed. At the same time, significant contributions will be made to environmental cleanliness by reducing nitrogen losses from agricultural areas.

The study determined the relationships between the readings obtained from the established plots and fertilizer rates. Therefore, the 4th reading period after fertilization (CGDD=928.9) was the most suitable for variable rate fertilizer application regarding NDVI variability. The experiment obtained $p<0.05$ statistically significant results between fertilization levels and yield and protein values. p values were determined as 0.001, 0.006, and 0.003, 0.025 for yield and protein for the 1st and 2nd years, respectively. According to the results, the seed varieties used in the experiment had statistically significant effects on yield and protein. Based on the results obtained at the trial location, the 4th fertilizer rate (16 kgN da^{-1}) in the 1st and 2nd years of the trials in terms of yield and the 5th fertilizer rate (20 kgN da^{-1}) in the 1st year and the 4th fertilizer rate (16 kgN da^{-1}) in the second year were determined as the appropriate fertilizer rate in terms of the highest protein content.

Conclusions

It was determined as 16.76 and 20.6 kg ha^{-1} for the Bezostaya and Ahmetağa varieties, respectively. When an economic analysis is made for the Haymana region where the study was carried out, the ton price of AN fertilizer is 815 TL, the size of the wheat cultivated land in Haymana Research and Application Farm is 570 da, and 20 kg of

pure nitrogen is applied to 1 decare. However, the amount that should be used is 16.76 kg. The difference is 3.24 kg of pure nitrogen, and since AN contains 33% nitrogen, 9.72 kg of AN is over-applied to 1 decare. For 570 decades, approximately 5540.4 kg more AN fertilizer is applied. Therefore, there is a loss of at least 45150.42 TL every year. As a result of this study, this loss can be prevented.

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Determination of Resistance of Winter Wheat Varieties Against Root and Crown Rot *Fusarium culmorum* Under the Artificial Drought Conditions

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ABSTRACT

This study was conducted to investigate the relationship between the recent increase in the frequency of drought conditions and Root-Crown Rot (*Fusarium pseudograminearum*, *Fusarium culmorum*) in rainfed wheat growing areas of Central Anatolia. In 2018, the experiment was established in the greenhouse of Konya Bahri Dağdaş International Agricultural Research Institute using 25 registered wheat varieties in a randomised block split-plot experimental design with 4 replications. Irrigation levels (100% field capacity and 50% field capacity) were designed as main plots, inoculation (+ and -) as subplots and varieties as sub-subplots. In the study, the response of the cultivars to inoculation under artificial drought conditions was evaluated by measuring Crown Score (CR), Lesion Length (LL), Number of Diseased Leaves from outside to inside (NDL) and Plant Height (PH) from five plants in each pot. Statistically, the differences between inoculation, irrigation, NDL and LL were found to be significant at P<0.0001 level, while the differences between varieties were found to be significant at P<0.001 level for the CR parameter. On the other hand, when the interactions were evaluated for the NDL parameter, the differences were found to be significant at P<0.0001 level for all three interactions of cultivar*inoculation, cultivar*irrigation and cultivar*inoculation*irrigation. When the effect of reduced water application intended to be used in resistance breeding was evaluated for 25 different varieties under *Fusarium culmorum* inoculation, there was an increase in CR from 41,7% (Gerek-79) to 487,5% (Altay-2000), NDL from 7,14% (Kirgiz-95) to 200% (Alpu-2001), LL from -36,84% (Karahan-99) to 283,33% (Altay-2000) and in PH reduction from 12,41% (Seval) to 32,22% (Kirgiz-95). The results showed that drought-stressed plants were already weakened and therefore more easily and severely infected by pathogens. According to these results, it has been determined that it is very important for the region to obtain resistance to drought and crown rot diseases, which have such an obvious relationship, in breeding studies.

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Introduction

The rapid growth of the world's population has a direct impact on the world and our Türkiye's nutritional needs. As the second most consumed crop in the world after rice and the main food source for around a third of the world's population, wheat is a strategic crop and ranks first among cultivated crops, accounting for about 221 million hectares planted. World wheat production is 776 million tons and the yield is about 351 kg/da (USDA, 2022). Among the agricultural areas of our country, wheat has the largest area under cultivation with 6.7 million ha and approximately 22 million tons of product are produced annually from these areas (TÜİK, 2022), placing our country among the top 10 wheat-producing countries in the world.

The importance of wheat as a strategic crop was better recognised during the COVID-19 pandemic and subsequent international crises. Wheat is grown almost everywhere in Turkey, but there are many barriers to production. Nearly 70% of the wheat production area in Turkey is rainfed. Therefore, it is inevitable that dry areas are extremely important for production and it is essential to develop drought-tolerant varieties. So, varieties developed by the Konya Bahri Dağdaş International Agricultural Research Institute, Türkiye's drought testing center, using successful breeding-physiology studies and new strategies, have entered the market in recent years and made a reputation.

Yields and economic losses due to diseases and pests in wheat production are also major issues. Rust diseases (*Puccinia* spp.) and soil-borne pathogens are an important group of pathogens in wheat production areas and are responsible for significant yield losses in wheat (Kınacı, 1992; Wildermuth and McNamara, 1994). Other important diseases affecting wheat production include root rot and root crown disease (*Fusarium pseudograminearum*, *Fusarium culmorum*), a major problem in the rainfed wheat production areas of central Anatolia. Studies show that

soilborne pathogens reduce wheat yields by 42-45% (Nicol, 2008; Arici et al., 2013).

While rust diseases are important because they can rapidly cause epidemics, Fusarium is one of the most important yield-losing diseases in drylands worldwide and in Türkiye (Cook, 1992; Burgess et al., 2001; Backhouse et al., 2004, Nicol et al., 2008). Given that this disease can cause more severe infections in dry conditions, studying these diseases is particularly needed in Central Anatolia, where wheat is grown most intensively. This pathogen can infect the roots, crown and internodes upwards, particularly when the plant is under water stress and has low water potential (Papendick and Cook, 1974). Moist conditions immediately after planting and dry conditions between flowering and hard ripening favour the development of the disease (Paulitz et al. 2002; Smiley et al. 1996). White head formation is a clear symptom of the disease. Effective water transport through the primordial root system is prevented by the fungus attacking the root and crown internodes. Also, the fungus is more effective when there is insufficient surface moisture and lateral roots are underdeveloped, especially in drought conditions towards the end of the season. This situation also prevents grain filling and development (Burgess et al., 2001).

Stubble burning, ploughing, crop rotation and chemical control are the methods used to control the disease, but they are insufficient. So, the use of resistant varieties to reduce the harmful effects of the disease is an alternative to these methods. Breeding studies are very timeconsuming and expensive. The basic principle of breeding is that to produce a good variety, the objective should be welldefined, the appropriate parents should be selected and the experimental technique should be well determined (suitable location and good observation). To this end, success in combination breeding is realised through the existence of the necessary variation resources and the efficient use of these resources. Knowing the heritability of the traits of the parents determined according to the purpose eliminates unnecessary combinations and gives an idea of the generation in which selection should start (Toklu and Yağbasanlar, 2005).

Taking into account the above studies and reasons, the fact that these factors are more frequent in our region, especially in areas severely affected by drought, and that diseases can cause more severe infections under stress factors, the idea that disease resistance studies should be carried out under artificial drought conditions and that drought and disease resistance traits should be found together in wheat varieties to be grown in production areas has come to the forefront.

Characterising the parents used in drought resistance breeding for their responses to disease will make it easier to develop varieties with more traits along with the right crosses, shortening this long-term process. Therefore, the study has been carried out under controlled conditions with 25 registered varieties currently in use and will be extended to more material according to the results obtained.

Material and Methods

Material

In this study, 25 bread wheat varieties developed by the TAGEM Institutes from national and international (IWWIP) programs were used as material (Table 1).

Table 1. Varieties used in the study and their characteristics.

No	Cultivars	Winter-Summer	Drought Resistance Status	Fusarium Resistance Status
1	Alpu-2001	Winter Bread Wheat	Susceptible	Susceptible
2	Altay-2000	Winter Bread Wheat	Moderate resistance (C)	Moderate resistance (C)
3	Çetinel-2000	Winter Bread Wheat	Susceptible	Susceptible
4	Bezostaya-1	Winter Bread Wheat	Susceptible	Susceptible
5	Gerek-79	Winter Bread Wheat	moderate resistance	Susceptible
6	İzgi-2001	Winter Bread Wheat	Susceptible	Susceptible
7	Kıraç-66	Winter Bread Wheat	Moderate resistance	Susceptible
8	Kırgız-95	Winter Bread Wheat	Susceptible	Susceptible
9	Kutluk-94	Winter Bread Wheat	Susceptible	Susceptible
10	Müfitbey	Winter Bread Wheat	Susceptible	Susceptible
11	Ahmetağa	Winter Bread Wheat	Susceptible	Susceptible
12	Dağdaş-94	Winter Bread Wheat	Moderate resistance (C)	Moderate resistance (C)
13	Ekiz	Winter Bread Wheat	Susceptible	Susceptible
14	Göksu-99	Winter Bread Wheat	Susceptible	Susceptible
15	Karahan-99	Winter Bread Wheat	Moderate resistance	Susceptible
16	Kınacı-97	Winter Bread Wheat	Susceptible	Susceptible
17	Konya-2002	Winter Bread Wheat	Susceptible	Susceptible
18	Bayraktar-2000	Winter Bread Wheat	moderate resistance	Susceptible
19	Demir 2000	Winter Bread Wheat	Susceptible	Susceptible
20	Gün-91	Winter Bread Wheat	Susceptible	Susceptible
21	İkizce-96	Winter Bread Wheat	Susceptible	Susceptible
22	Seval	Winter Bread Wheat	Susceptible	Susceptible
23	Tosunbey	Winter Bread Wheat	Susceptible	Susceptible
24	Uzunyayla	Winter Bread Wheat	Susceptible	Susceptible
	Yakar-99	Winter Bread Wheat	Susceptible	Susceptible

Methods

The experiments were conducted as pot trials in the fully controlled greenhouses in the Bahri Dağdaş International Agricultural Research Institute (BDUTAE) using 25 registered wheat varieties in a randomised block split-plot experimental design with 4 replications. Irrigation levels (100% field capacity and 50% field capacity) were designed as main plots, inoculation (+ and -) as subplots and varieties as sub-subplots.

First, wheat seeds were surface sterilized and germinated in an incubator. The seeds were first soaked in 98% ethyl alcohol for 5 minutes, then in 4.5% sodium hypochlorite solution for 1 minute, and then washed 3 times with sterile distilled water. To germinate the seeds, filter paper moistened with sterile water was placed on 9 cm diameter Petri dishes, and seeds were placed on top and incubated at 23 °C for 4 days. The 5kg pots used in the experiment were filled with soil (20% sand, and 80% soil). Pre-germinated seeds were placed in the pots with pliers, with 10 plants in each pot, then covered with the soil mixture. After the growing of the plants, the number of plants in each pot was reduced to five.

The inoculum was prepared as described by Nicol et al. 2008, and the isolate was grown in Petri dishes on PDA for 2 weeks. The isolates were then placed in moistened and autoclaved propylene bags filled ¼ full with wheat and left to develop conidia for 2-3 weeks at 23°C. Distilled water was added to the bags and the spore suspension was filtered through cheesecloth for particle removal. The resulting dense spore suspension was used for the inoculation of seeds using a thoma slide and diluted to a density of $1x10^6$. The prepared inoculum was applied to the root collar and soil using a micropipette at 100 µl 1 week after the seeds germinated (Mitter et al., 2006; Erginbaş et al., 2008; Özdemir, 2014).

To create artificial drought conditions, the field capacity of the experimental plants (pots) was first measured to obtain different water contents (100% field capacity and 50% field capacity) and the watering time was specified for all pots to determine the disease effect under dry conditions. The pots were then weighed at 5-day intervals throughout the study and the water deficit pots were replenished to the determined field capacity (for 100% irrigation) or half (for 50% irrigation).

Greenhouse conditions were set at 25±2°C with 16 hours of light and 8 hours of darkness, and plants were uprooted after 8 weeks. Crown score (CS), lesion length (LL), number of diseased leaves from outside to inside (NDL) and plant height (PH) were measured from five plants in each pot at uprooting to assess the response of the cultivars to inoculation under artificial drought conditions. CS, after washing the root and crown parts in water, was scored on a scale of 0 to 10 at 10 cm discoloration of the crown (0= No discoloration, 1= 1-4%; 2=5-9%; 3=10-19; 4=20-29; 5=30-39; 6=40-49; 7=50-59; 8=60-69; 9=70-79; 10=80 and more). To evaluate the inoculation, re-isolation studies were performed. Classical phytopathological methods were used to isolate and identify the fungi from samples taken from the medium.

Results and Discussion

Statistically, the differences between inoculation, irrigation, NDL and LL were found to be significant at $P<0.0001$ level, while the differences between varieties were found to be significant at $P<0.001$ level for the CS parameter. On the other hand, when the interactions were evaluated for the NDL parameter, the differences were found to be significant at $P<0.0001$ level for all three interactions of cultivar*inoculation, cultivar*irrigation and cultivar*inoculation*irrigation.

In the study to determine the disease resistance of varieties under drought stress induced by reduced watering, different results were obtained depending on the parameters. However, compared to 100% water treatment, CS and NDL increased in all cultivars, LL increased in all cultivars except Izgi-2001, Kutluk-95, Kirgiz-94 and Karahan-99, and PH decreased in all cultivars in 50% water treatment (Figure 1,2,3,4, 5, 6, 7, 8).

When Figure 1 examined with these statistical analysis data, it is noticable that the plants in the 50% water deficit block were divided into two sub-subjects inoculated and uninoculated, and in the inoculated sub-group plant development was weaker and yellowing was highly evident. This can be explained by the fact that deficit irrigation conditions create a stress situation in favour of the pathogen, allowing the pathogen to act more severely and symptoms to become more pronounced. These results are very similar to Papendick and Cook (1973), Cariddi and Catalano (1990) and Alahmad et al., (2018). However, Figure 2 shows that although the full irrigation block was divided into two sub-blocks with and without inoculum, the effect of the disease was not as marked as in the 50% water restriction block, and because there was no water restriction, the pathogen caused fewer symptoms in the plants due to the absence of drought stress conditions. These visual assessments are described in detail with the parameters CS, LL, PH and NDL taken at harvest.

Figures 3 and 4 indicate that, depending on the amount of irrigation and the inoculation conditions, there are apparent differences in plant height, volume, colour and development in plants with different genetic characteristics (developed for dryland and irrigated condition). In Figure 3, it is clear that the Bezostaya-1 variety, developed for relatively irrigated areas, is very strongly affected by water deficit (100%-50% comparison), and the difference becomes even more distinct when the effect of the disease is combined with the conditions (inoculated-uninoculated comparison under 100% irrigation or inoculated-uninoculated comparison under 50% irrigation). In Figure 4, Karahan-99, one of the most drought-tolerant cultivars, could tolerate water restriction (100% water inoculated vs. 50% water inoculated), but could not tolerate inoculation under the same irrigation conditions because it was not resistant to the disease (inoculated vs. uninoculated under 100% irrigation and inoculated vs. uninoculated under 50% irrigation).

In addition to the different responses of irrigated and drought-tolerant cultivars to the effect of reduced water application on CS, different reactions were observed in drought and disease tolerant cultivars.

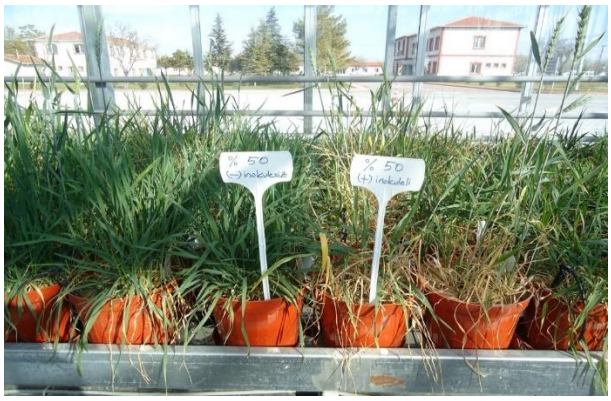

Figure 1. View of pots with and without inoculum in the reduced water block of the greenhouse study. Although the irrigation level (50% water restriction) was the same, it is easy to see the yellowing of the plants and the reduction in volume due to the effect of the disease.

Figure 2. View of pots with and without inoculum in the full field capacity treatment block in the greenhouse study. Although the watering level (100%) was the same, the yellowing of the plants and reduction in volume can be seen due to the effect of the disease.

Figure 3. Bezostaya-1 cultivar under full (100%)-limited irrigation (50%) and Fusarium culmorum inoculated-non-inoculated conditions.

Figure 4. Karahan-99 cultivar under full (100%)-limited irrigation (50%), Fusarium culmorum inoculated-non-inoculated conditions.

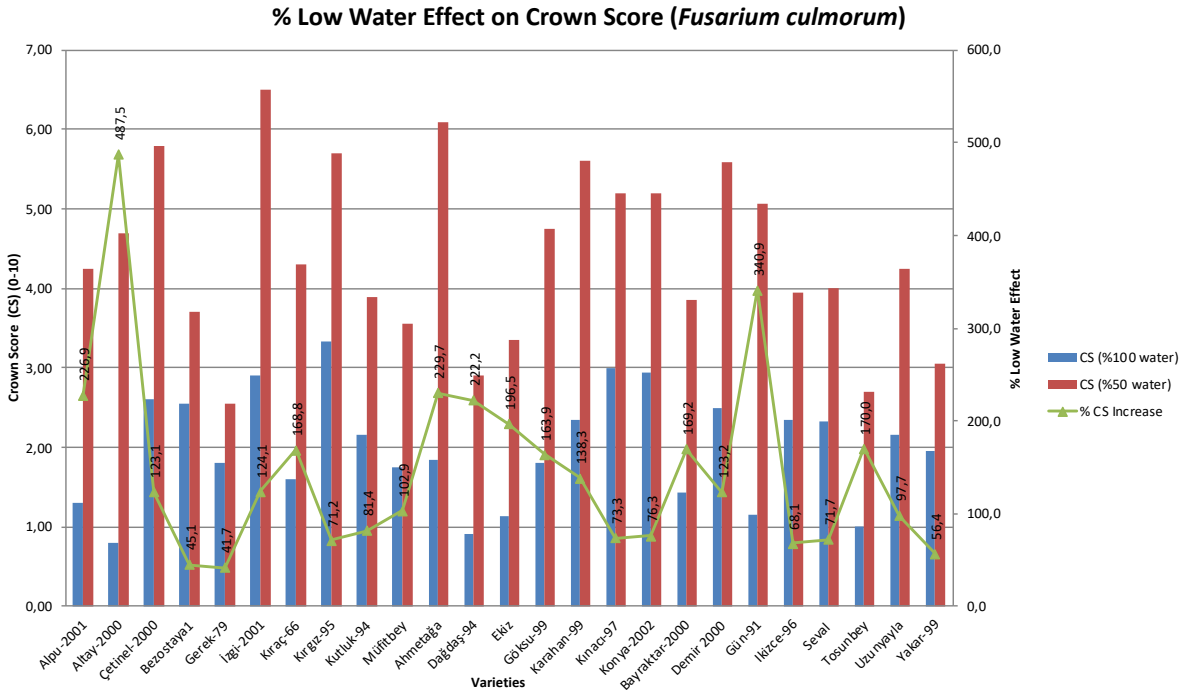

Figure 5. Evaluation of CS in wheat varieties at 50% and 100% irrigation and % change in CS according to irrigation difference

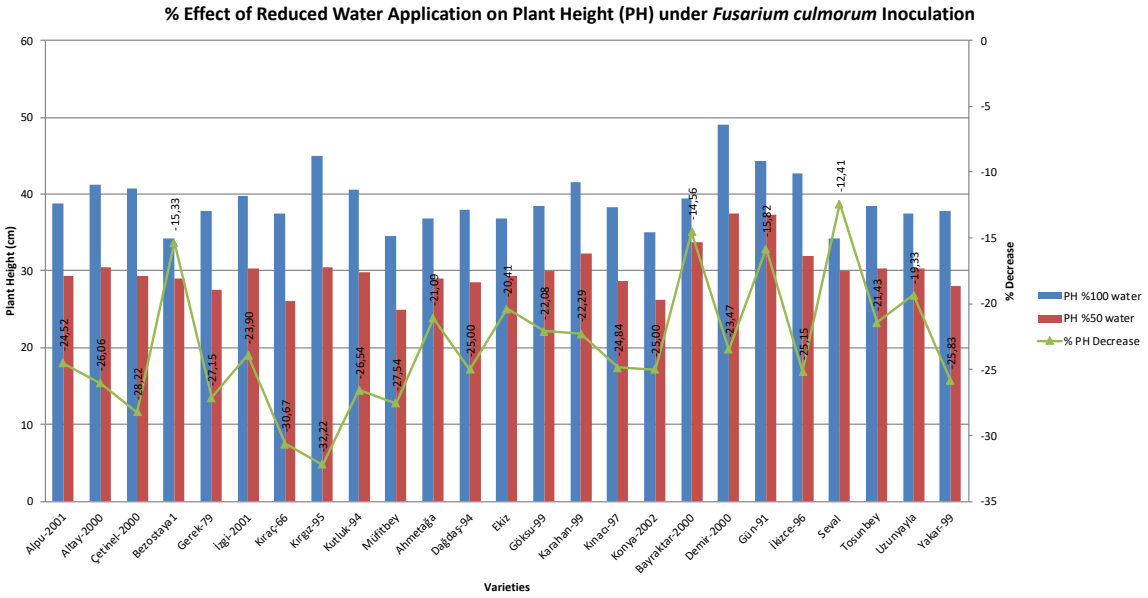

Figure 6. Percentage effect (%) of reduced water application (50% and 100% irrigation) on PH under Fusarium culmorum inoculation

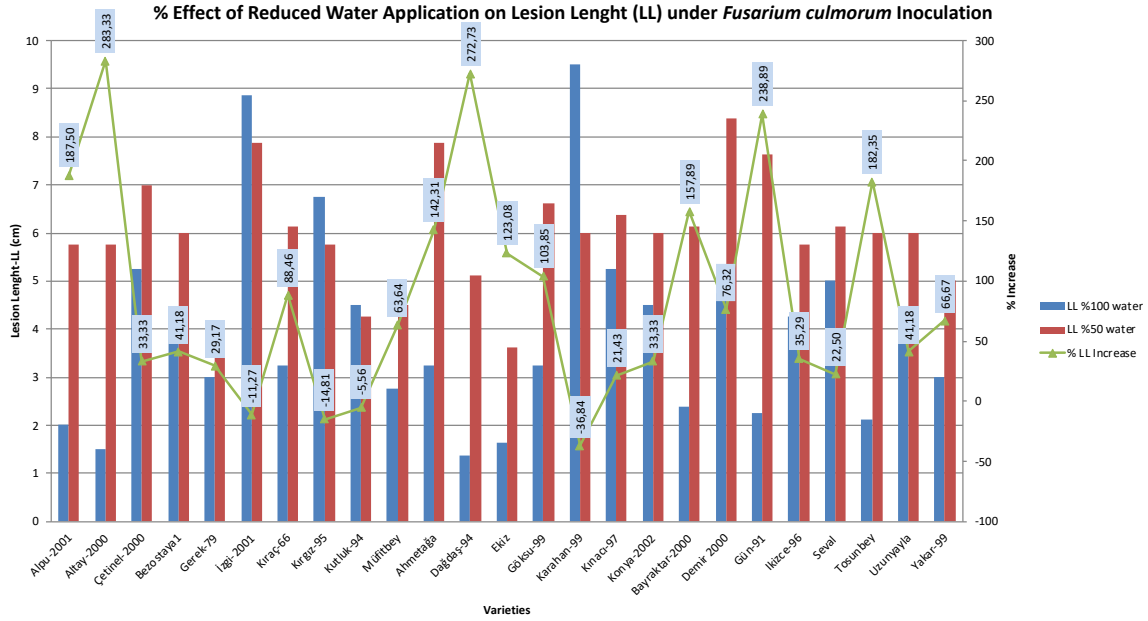

Figure 7. Percentage effect (%) of reduced water application (50% and 100% irrigation) on LL under *Fusarium culmorum* inoculation

Altay-2000 and Dağdaş-94 were used as drought and disease tolerant cultivars and compared to other cultivars, especially Dağdaş-94 suppressed and tolerated disease more than other cultivars at 50% and 100% irrigation levels and limited disease symptoms (0-10 scale). In % disease change rates, the varieties with the highest increase in disease score with decreasing irrigation level were Altay-2000 (487,5%), Gün-91 (340,9%) and Ahmetağa (229,7%), while the varieties with the lowest increase in disease score were Gerek-79 (41,7%), Bezostaya-1 (45,1%), and Yakar-99 (56,4%), respectively (Figure 5). The results revealed that Altay-2000, used as a control for drought and disease tolerance, did not provide the expected resistance to drought stress and was even the variety with the highest proportional increase in disease symptoms. However, when CS values are analysed in both conditions, it stands out as one of the varieties with the lowest total CS of all varieties. The overall CS scores (%100 CS+ %50 CS) show that varieties with high drought tolerance such as Tosunbey (3,70), Dağdaş-94 (3,80), Ekiz (4,48), Yakar-99 and Bayraktar-2000 are at the forefront.

The effect of reduced water application on PH under *Fusarium culmorum* inoculation showed that the reduction in PH of the varieties was more similar to each other compared to CS, ranging from a minimum of 12,41% to a maximum of 32,22%. Drought and disease tolerant varieties Altay-2000 and Dağdaş-94 showed 26,06% and 25% reductions in plant height respectively; the highest reduction in PH was observed in Kirgiz-95 (32,22%), Kiraç-66 (30,67%), Çetinel-2000 (28,22%) and the lowest reduction in PH was observed in Seval (12,41%), Bayraktar-2000 (14,56%) and Bezostaya-1 (15,33%) (Figure 6).

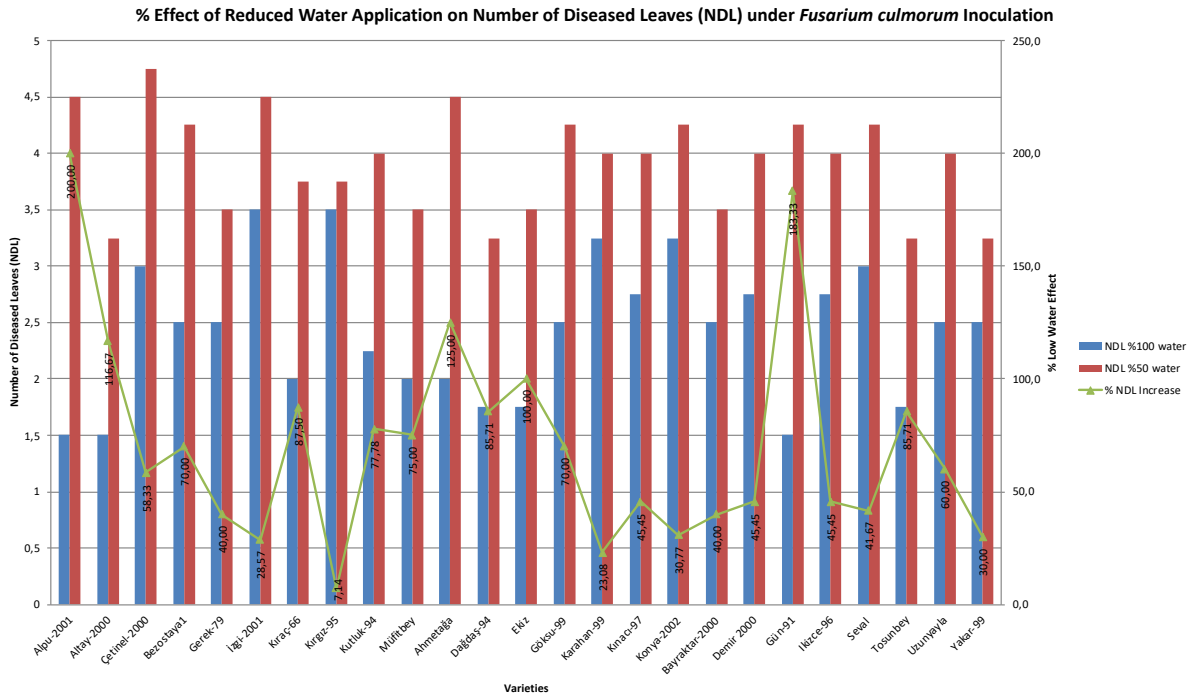

Figure 8. Percentage effect (%) of reduced water application (50% and 100% irrigation) on the NDL under Fusarium culmorum inoculation

The percentage effect of *Fusarium culmorum* inoculation with reduced water application on LL was observed to increase in almost all cultivars, while a decrease was observed in Karahan-99, İzgi-2001, Kırgız-95 and Kutluk-94. The varieties with the highest increase in LL were Altay-2000 (283,33%), Dağdaş-94 (272,73%) and Gün-91 (238,89%); the varieties with the lowest increase were Kınacı-97 (21,43%), Seval (22,50%) and Gerek-79 (29,17%). In addition to all these evaluations, although the control varieties Altay-2000 and Dağdaş-94 showed the highest increase in lesion rate, these varieties were among the lowest-scored varieties in CS and total CS. As a result, it can be concluded that disease tolerance was maintained and LL was suppressed under irrigation and limited irrigation conditions (Figure 7).

When examining the effect of artificial drought with reduced water application on NDL under disease stress, it was found that the number of diseased leaves increased from outside to inside in all varieties under artificial drought. Under full and semi-irrigated conditions, Alpu-2001 (200%), Gün-91 (183.33%) and Ahmetağa (125%) showed the highest increase in NDL, while the lowest increases were observed in Kırgız-95 (7,14%), Karahan-99 (23,08%) and İzgi-2001 (28,57%). In evaluating the number of diseased leaves, it can be stated that the control varieties Altay-2000 and Dağdaş-94 had the lowest values in terms of NDL in both conditions, except for the % change, and this situation is due to the disease and drought tolerance of the varieties (Figure 8).

Conclusion

The results showed that although all the varieties in the study had different characteristics (red-white grain, developed for dryland-irrigated areas, tall-short PH, earliness-lateness, drought tolerant, tolerant to *Fusarium culmorum*, etc.), it was observed that CS, LL and NDL increased and PH shortened in all varieties inoculated under reduced water application. Considering the overall study, this can be explained by the fact that the plants exposed to drought are already weakened under stress and therefore more easily and severely infected by pathogens as previous studies like Papendick and Cook (1973), Cariddi and Catalano (1990) and Alahmad et al., (2018). Decreases in yield factors such as plant growth, volume and PH under limited irrigation conditions (artificial drought, disease and combination) are extremely important for wheat production. The fact that agricultural production, which has become problematic as a result of climate change, is also affected by disease means that the problem of production will be exacerbated. These findings highlighted the importance of studying resistance to multiple biotic and abiotic factors together under the same conditions (especially for Central Anatolia) in breeding and resistance breeding studies. The combination of resistance to drought stress and root-crown rot diseases was found to be particularly important for the region, as they are connected.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Fatih Özdemir: Validation, Writing - original draft, Methodology, Investigation, Conceptualization, Validation, Review and editing, Methodology, Investigation, Conceptualization, Validation, Writing - original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Formal analysis, Data curation, Formal analysis, Data curation.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Analysis of Monthly Precipitation at the Basin Scale in Türkiye

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ABSTRACT

Basin-based water management strategy is one of the necessary instruments for the protection and sustainable use of water resources against climate change. In this paper, the monthly precipitation distributions of the 25 major basins in Türkiye were produced, and amounts and volumes were computed and analyzed. Only annual modeling and assessments of precipitation may hide months with precipitation shortages. Empirical Bayesian Kriging (EBK), Ordinary Kriging (OK), and Inverse Distance Weighting (IDW) were implemented in interpolation. EBK outperformed in all months and calculations were based on the EBK. The month with the highest precipitation potential in Türkiye is December (77.9 mm, 60.77 billion m^3), and the month with the lowest precipitation potential is August (13.8 mm, 10.76 billion m^3). In the basins, the monthly precipitation amounts range between 2.7 mm and 185.2 mm, and the volumes range between 0.02 billion m^3 and 13.24 billion m^3. The basins with the highest precipitation depth were determined as the East Black Sea, Antalya, Asi, and Ceyhan, and the lowest as the Small Menderes, Konya, and Tigris-Euphrates in different months. The monthly precipitation patterns and potentials of the basins vary widely. In May, June, July, August, and September, when water, particularly agricultural irrigation, is required the most, the 20 basins, except for the 5 located in Northern Türkiye precipitation shortage was determined.

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Introduction

Precipitation is the major source of freshwater and is the key input for calculating the water potential and water policies of a watershed or a country. In many studies, particularly on sustainable use of water resources, agricultural irrigation, floods, drought and watershed management, and climate change, determining the precipitation model as close to reality as possible is crucial. Accurate rainfall information is vital for decision-makers. Rainfall is an intermittent climatic element, with major temporal and spatial instability and discontinuity. Therefore, a more frequent measurement network than the other meteorological parameters is required for precipitation. Precipitation data can be obtained from meteorological satellites and radars as well as rain gauges (Akgül and Aksu, 2021). Rain gauge-based rainfall measurements are the best for their accuracy. The precipitation measurement is performed at a certain place and represents that specific location. It is not possible to set up stations at any desired location because of financial and geographical limitations. Spatial precipitation data obtained from point precipitation data via interpolation methods are used in hydrological practice (Goovaerts, 1997; Ly et al., 2013)

There are two requirements for modeling an accurate precipitation distribution map and calculating the potential: First, an optimum raingauge network, which can best represent the study area; second, the optimum interpolation method. The use of non-homogeneous, inadequate, and off-target precipitation data causes numerous difficulties. The accuracy of rainfall estimation increases as the number of raingauges increases; however, fewer raingauges eventually decrease the accuracy of areal precipitation estimations and increase estimation errors (Berne et al., 2004; Cheng et al., 2012; Shehu and Haberlandt, 2021). In this respect, the World Meteorological Organization (WMO) suggests that the observation data should cover at least 30 years, and in regions such as Türkiye, there should be at least one raingauge every 2500 km^2 (WMO, 2008). As per this suggestion, there should be at least 312 raingauges in Türkiye, which has 780,000 km^2 of surface area.

It was reported in many studies conducted using precipitation data in Türkiye that the frequency and homogeneity of precipitation stations were not at the desired level (Kadioglu et al., 1999; Çıtoğlu et al., 2017; Raja et al., 2017; Aksu, 2020; Aksu, 2021).

Turkish State Meteorological Service (TSMS) is the foundation that observes all climate elements and provides data for studies in this field. TSMS has 254 stations of usable quality. These stations are centered around inhabited areas and are not homogeneously distributed over the country. Since the early 2000s, Automated Weather Observing System (AWOS) has been set up at more than 2000 locations, and precipitation observations have been recorded. These stations cannot yet be used in studies because of inadequate data periods (at least 30 years). On the other hand, Turkish State Hydraulic Works (TSHW), which is the foundation in charge of hydraulic works in Türkiye, operates water-shed-based precipitation and evaporation observations around dams and ponds. TSHW has 137 precipitation stations of usable quality. The rural TSHW stations and the urban TSMS stations complement each other. In this study, TSHW stations were used in addition to TSMS stations.

Spatial interpolations are mathematical equations or methods, which estimate data in unmeasured areas by using the data of measured areas. There are various interpolation methods. Implementation of a suitable interpolation method varies from region to region. The selection of the method depends on factors, such as the aim of the study, the density of stations, and the topography of the area. The density of the station network affects the performance of interpolation. In areas, where the observation network is dense, interpolation methods show similar spatial distribution (Borges et al., 2016; Frazier et al., 2016)

There are many studies in the literature, in which deterministic and geostatistical methods for precipitation data interpolation are used in various areas. Studies are centered around comparisons of methods to find the most suitable method (Sun et al., 2015; Borges et al., 2016; Antal et al., 2021; Das, 2021).

There are papers in Türkiye, in which deterministic and geostatistical techniques were used or compared for the interpolation of rainfall data (Türkoğlu et al., 2016; Çıtoğlu et al., 2017; Raja et al., 2017; Aksu, 2021; Aksu, 2023)

Monthly rainfall data provide more correct intra-yearly rainfall knowledge than annual and seasonal rainfall data. Only annual assessments of precipitation distribution may hide months of the year with precipitation shortages. Monthly rainfall information is an element that makes significant additions to the monitoring of drought and climate change, basin and water resources management, and agricultural and hydrological practices. Although there are many studies investigating the annual and seasonal potential and variability of precipitation in Türkiye (Kadıoğlu et al., 1999; Türkeş et al., 2009; Çiçek and Duman 2015; Raja et al., 2017; Türkeş, 2020a; Aksu, 2021, Aksu, 2023). There is no study in the literature explaining the watershed-based monthly potential and spatial distribution of precipitation.

This study aims to determine and analyze the monthly precipitation potentials and models of the 25 major basins in Türkiye. Therefore, geostatistical EBK and OK, and deterministic IDW techniques were implemented in the GIS environment. Results were compared through the cross-validation method. In the work, different from the earlier research in Türkiye, monthly precipitation data from two foundations (TSMS and TSHW) were joined to fulfill the rain gauge network intensity recommendation of the WMO. The location and intensity of the rain gauge network influence the accuracy of precipitation estimation (Borges et al., 2016; Frazier et al., 2016)

With its semi-arid precipitation regime, and 1500 m^3 of yearly water amount per individual, Türkiye is among the water-scarce countries (Falkenmark 2013; Aydın et al., 2017; Türkeş, 2020a). Water consumption in Türkiye has increased by approximately 40% within the last 20 years (Aksu, 2021). More than 70% of the water supply is used for irrigation purposes in Türkiye (MAF, 2019). Basin-scale water administration is one of the necessary instruments for the protection and sustainable use of water resources against drought that may happen depending on climate change and ever-growing water demand (WFD, 2000). This paper is crucial for watershed administration since Türkiye has been working on an integrated watershed-scale water administration model for adaptation to climate change. At the same time, this study is also important in monitoring monthly precipitation changes at the basin scale depending on climate change as Türkiye is situated within the Mediterranean and Middle East regions, which are largely affected by climate change (WWDR, 2020; Türkeş, 2020b; WWDR, 2021).

Materials and Methods

Study Area and Data

Türkiye is situated in the Mediterranean basin between 26-45^0 eastern meridians and 36-42^0 northern latitudes and is surrounded on three sides by the sea (Figure 1). Marina climate and precipitation can penetrate the inner basins of the country by weakening since The Taurus Mountains at the south and the Black Sea Mountains at the north lay parallel to the seaside. Because of the large number of tectonic graben horst systems extending in an east-west direction in Western Türkiye, rainfall can penetrate the interior parts of this area. The hills facing the Black Sea in the North and the Mediterranean Sea in the South are the zones of the country with the most precipitation. Altitude in Türkiye goes up to 5137 meters above sea level, and the average altitude is around 1100-1200 meters. The average altitude gradually increases from west to east. Because of the unstable topography of the country, major precipitation differences may be observed over short distances.

Türkiye has 25 major watersheds. The watersheds covering the greatest area are Tigris-Euphrates (175,882 km^2), followed by Kızılırmak (82,082.5 km^2), Sakarya (63,242.9 km^2), and Konya (49,805.3 km^2) watersheds, respectively. There are twelve watersheds covering between 20,000 and 30,000 km^2 of area. Burdur (6,273.8 km^2), Small Menderes (7,027.1 km^2), Asi (7,904.2 km^2), Akarçay (7,954.5 km^2), and North Aegean (9,963.6 km^2) are watersheds covering smaller areas.

While 17 watersheds are located by the sea, 8 watersheds are landlocked areas. The landlocked watersheds are Akarçay, Burdur, and Konya in Central Anatolia, Tigris-Euphrates, Van, Aras, and Çoruh in Eastern Anatolia, and Meriç-Ergene in Northwest Anatolia. Tigris-Euphrates, Asi, Çoruh, Aras, and Meriç-Ergene are transboundary watersheds. Tigris-Euphrates, Aras, and Çoruh are upstream watersheds; Asi and Meriç-Ergene are downstream watersheds.

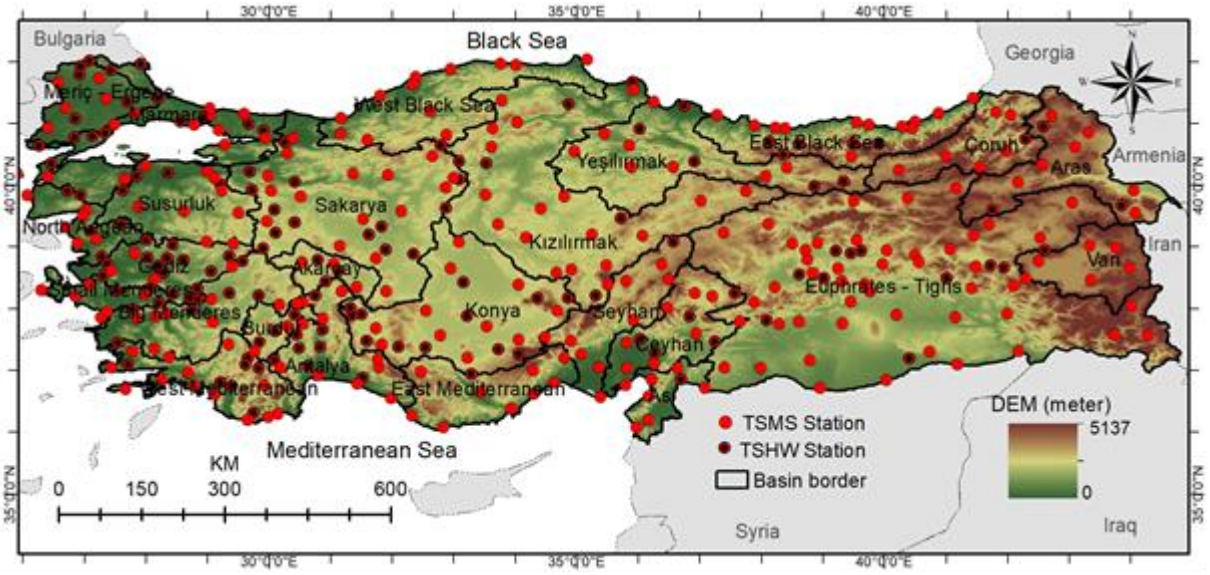

Figure 1. Rain gauge station network and watersheds on an elevation model of Türkiye

The annual precipitation amount of Türkiye is 587 mm and this value varies between 1013 mm (East Black Sea) and 389 mm (Konya) in the watersheds (Aksu, 2021). Antalya (939 mm), West Mediterranean (834), and Asi (800 mm) are the wettest watersheds of Türkiye after the East Black Sea watershed. In the 7 watersheds, the annual rainfall amount is below 500 mm.

In the study, the monthly precipitation data of 391 raingauges (254 of which are operated by TSMS and 137 by TSHW) for the period between 1965 and 2018 were used. Figure 1 shows the watersheds in Türkiye and the locations of TSHW and TSMS raingauges.

Methodology

The interpolation technique is a method used for estimating values at unmeasured locations by using the data of points, where measurements of variables were conducted. Variables in neighboring locations give more similar results than remotely located variables. The geostatistical EBK and OK, and deterministic IDW techniques were used in the study for areal interpolation of monthly precipitation data. Geostatistical analyst tools of the Geographic Information Systems (GIS) program ArcGIS 10.8 were used for the implementation of interpolation methods and to create an areal distribution map of monthly precipitation data. *Ordinary Kriging (OK)*

OK is the most widely used Kriging technic (Goovaerts, 1997; Ly et al., 2013). In the OK method, first, an experimental semi-variogram analysis of the observation data is performed. Then theoretic semi-variogram model is adapted (fitted) to this structure. The theoretic semi-variogram is obtained by applying an algebraic function to the experimental semi-variogram. Spherical, circular, and Gaussian variogram models are widely used in precipitation data. Gaussian and circular models were the most suitable theoretic models for this study. The formulas of the experimental semivariance, the Gaussian, and the Circular theoretical models are shown respectively below (Webster and Oliver, 2017):

$$\gamma(h) = \frac{1}{2N\,(h)} \sum_{i=1}^{N(h)} [Z(X_{m_i}) - Z(X_{m_i} + h)]^2 \quad (1)$$

$$\gamma(h) = C_0 + C_1 \left[1 - \exp\left(\frac{-3h^2}{a^2}\right)\right] \; h \geq 0 \quad (2)$$

$$\gamma(h) = \begin{cases} C_0 + C_1 \left[1 - \frac{2}{\pi} cos^{-1}\left(\frac{h}{a}\right) + \frac{2h}{\pi a}\sqrt{1 - \frac{h^2}{a^2}}\right] h \leq a \\ C_0 + C_1 \; h > a \end{cases} \quad (3)$$

$\gamma(h)$: function of semi-variogram, hinge on lag or h interval; h: interval vector; $Z(X_{me,i})$: measured rainfall of every i location; $Z(X_{me,i} + h)$: measured rainfall interval h from i location; $N(h)$: data pairs number; C_1: partial sill; C_0: nugget effect; a: range. Kriging, which is the fundamental instrument of geostatistics, is the best linear unbiased estimator. Its ability to calculate the variance value for each estimated point is the defining characteristic of this technique. Estimation is performed with the weighted average of the measured value. The weightings are based on the distance and the model variogram. The basic formula used in OK is given as equation (4) (Webster and Oliver, 2017).

$$\hat{Z}_{OK}(X_0) = \sum_{i=1}^{N} W_i^{OK} Z(X_{me,i}) \quad (4)$$

$\hat{Z}_{OK}(X_0)$: estimated OK rainfall value at X_0 location; W_i^{OK}: weight for each $Z(X_{me,i})$ location; $X_{me,i}$: measuring point; N, shows the number of locations used in OK estimation. After the theoretical semivariogram structure of the investigated variable (precipitation) is determined mathematically (equations 2, 3), the values of the unobserved points in the study area are estimated with the OK (equation 4) technique.

Empirical Bayesian Kriging (EBK)

Geostatistical EBK is a reliable and simple way for automatic interpolations of variables (Li et al., 2022). EBK does not require its users to manually regulate parameters to get accurate outcomes (Gribov and Krivoruchko, 2020). Classical Kriging methods compute the semi-variogram from observed data points and utilize this single calculated semi-variogram to make predictions at unknown points. After this process, the predicted semi-variogram is assumed to be the true semi-variogram for the interpolation area. Unlike classical Kriging methods, EBK uses many

semi-variogram models and takes account of the errors that happen when estimating the semi-variogram models. Therefore, this process of EBK causes standard estimation errors to be more accurate than traditional Kriging techniques (Zou et al., 2021). EBK semi-variogram prediction process requires the steps below (Krivoruchko, 2012):

1. A semi-variogram model is estimated from the measured data.
2. Using the estimated semi-variogram model, new data at each input point is simulated.
3. A new semi-variogram model is predicted using the simulated data. Using Bayes' rule, the weights of the new semi-variogram are computed. Bayes' rule measures the likelihood of a predicted semi-variogram to simulate observed data.

Inverse distance weighted (IDW)

The IDW is frequently used in the spatial interpolations of many variables, especially climate parameters. The weight of precipitation values measured at a short distance has a greater influence on the predicted rainfall values than those measured at long distances (Borges et al., 2016). The general equation of IDW is as follows:

$$R_{p_j} = \frac{\sum_{j=1}^{N} \frac{1}{d_i^P} R_{O_j}}{\sum_{j=1}^{N} \frac{1}{d_i^P}} \quad (5)$$

N is the number of rainfall observation locations used for the prediction. R_{p_j} is the rainfall to be predicted. R_{O_j} are the observed rainfall values. d_i are distances between R_{p_j} and R_{O_j} points. P is the power parameter. Weightage degrees of observed values are controlled by exponent power. The weight of the precipitation values of the close neighbors in the interpolation increases as the power value increases.

Cross-validation

Cross-validation is a method, which is frequently used for the evaluation and comparison of spatial interpolations. By the method, the relations between measured and estimated values are examined. For this purpose, the rainfall value of one station is temporarily separated from the data set. The rainfall value of the separated point is estimated by utilizing the rainfall data of the remaining raingauges. This application is implemented in each rainfall station. The error margin between the measured and estimated precipitation value is measured. Various error measurement methods are used in the evaluation of produced data (Frazier et al., 2016; Antal et al., 2021). In this study, Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and Determination Coefficient (R^2) were used. MAE and RMSE measures are widely applied because of their theoretical relevance in statistical modeling (Bagirov et al., 2017). RMSE shows the size of the error. Since the weight of major errors is greater, they are sensitive to extreme values. MAE gives a mean error estimate, and it is not affected by extreme values. Low MAE and RMSE values show verisimilitude of the estimated precipitation values. R^2 represents the relationship between observation and estimated values. It indicates the power of the linear relation. The formulas for these techniques are presented below:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|V_{m_i} - V_{e_i}\right| \quad (6)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(V_{m_i} - V_{e_i})^2} \quad (7)$$

$$R^2 = \left\{ \frac{\sum_{i=1}^{n}\left(V_{m_i} - \bar{V}_m\right)\left(V_{e_i} - \bar{V}_e\right)}{\left[\sum_{i=1}^{n}\left(V_{m_i} - \bar{V}_m\right)^2\right]^{1/2}\left[\sum_{i=1}^{n}\left(V_{e_i} - \bar{V}_e\right)^2\right]^{1/2}} \right\} \quad (8)$$

V_m: measured rainfall value; V_e: estimated rainfall value; $\bar{V}_m$: average of measured rainfall values.

Results

The addition of 137 TSHW raingauges to 254 TSMS raingauges has increased the number of available raingauges by 54%. The area represented by 1 raingauge has been increased from 3071 km^2 to 1995 km^2. Therefore, WMO's standard of at least one raingauge in every 2500 km^2 in topographies like Türkiye has been achieved.

Table 1 indicates the statistical results of the monthly rainfall data of 391 observation points in Türkiye. The maximum precipitation was found to be 324.4 mm in December, and the minimum was 0 mm in July and August. The mean monthly precipitation values of these stations range between 16.1 mm in August and 92.9 mm in December. When evaluated together with other statistical data, it was found that precipitation in Türkiye showed great differences within and between months.

The monthly average precipitation histograms of the stations are demonstrated in Figure 2. The monthly mean rainfall is below 16 mm at 269 stations in July, 19 mm at 307 stations in August, and 29 mm at 304 stations in September. On the other hand, the average precipitation is over 300 mm at 1 station in January, and at 2 stations in October and December. The stations with lower precipitation levels have higher frequencies; the stations with higher precipitation levels have lower frequencies.

In order to determine the optimum model for each interpolation method, various parameters, such as power coefficient, neighborhood, sector, transformation, and semi-variogram, were tested until a minimum error rate was obtained. In the IDW method, the optimum power coefficient ranged between 2.04 and 3.93 (Table 2). Figure 3 shows the experimental and theoretical models and parameters, which ensure optimum monthly results for the OK method. In the EBK method, empirical and log empirical were used as transform types, and whittle-detrended and K-Bessel detrended were used as a semi-variogram model (Table 3).

When the monthly precipitation distribution models derived were analyzed, it was found that the OK (Figure 4), EBK (Figure 5), and IDW (Figure 6) methods created similar patterns. As expected, the bull's eye effect was observed around the measuring points on the maps attained through IDW. The distribution maps obtained through OK and EBK formed a precipitation pattern in concordance with the topography.

The errors and performance results revealed through cross-verification applied to compare OK, EBK, and IDW techniques, are presented in Table 2. In IDW, R^2 was found between 0.52 (April) and 0.85 (August), RMSE was found between 8.15 (July) and 31.22 (December); MAE was found between 4.24 and 21.9. In OK, R^2 was found between 0.56 (April) and 0.87 (July and August), RMSE was found between 7.4 (July) and 29.06 (December); MAE was found between 3.8 and 20.42. In EBK, R^2 was found between 0.64 (April) and 0.92 (August), RMSE was found between 6.43 (July) and 26.03 (December); MAE was found between 3.41 and 17.83. According to these comparison results, EBK outperformed OK and IDW in all months. Similarly, OK outperformed IDW.

Table 1. Descriptive statistics of monthly mean rainfall data in Türkiye (mm)

Months	Minimum	Maximum	Mean	Std. Dev.	Skewness	Kurtosis	First Quartile	Median	Third Quartile
January	14	312.5	84.1	52.2	1.4	5.2	43.9	73.8	110
February	14.1	232.8	69.6	39.2	1.1	4.3	38	63.9	89.6
March	21.4	194.4	65.8	28.3	1.1	4.6	44.6	60.4	80.5
April	25.4	161.7	60.0	20.3	1.7	6.5	47.1	54.9	66.2
May	14.2	114.2	50.0	16.8	0.8	4.1	38.8	48.1	59.2
June	1.8	161.6	31.9	22.0	2.1	10.7	18.1	27.5	40.5
July	0	160.1	16.5	20.4	3.6	20.9	4.8	11.3	19.4
August	0	187.1	16.1	24.5	4.2	25.5	4.1	9.1	15.5
September	0.7	282.9	25.4	30.9	4.6	30.7	11.6	16.6	26
October	18.1	317.5	56.9	37.3	3.5	20.4	36.5	46.3	64.4
November	18	255.9	73.0	39.8	1.4	6.0	41.9	65.4	92.6
December	14	324.4	92.9	55.8	1.3	4.9	47.7	81.7	119.8

Table 2. Monthly error and performance metrics of the methods

	EBK			OK			IDW		
Months	MAE	RMSE	R^2	MAE	RMSE	R^2	MAE	RMSE	R^2
January	17.09	24.74	0.813	18.88	27.10	0.749	20.30	28.79	0.722
February	13.96	20.03	0.795	15.27	21.85	0.738	16.35	23.19	0.705
March	11.60	16.08	0.721	12.69	17.86	0.655	13.46	18.66	0.622
April	8.77	12.31	0.638	9.72	13.59	0.557	10.05	14.16	0.524
May	6.20	8.68	0.766	7.33	10.13	0.664	7.39	10.32	0.634
June	5.31	8.26	0.894	6.16	9.43	0.85	6.47	9.75	0.81
July	3.41	6.43	0.906	3.80	7.40	0.873	4.24	8.15	0.841
August	3.49	7.23	0.917	4.14	8.94	0.872	4.36	9.62	0.848
September	4.56	10.61	0.901	5.75	12.45	0.846	6.48	14.67	0.831
October	8.34	14.14	0.857	9.71	16.84	0.797	11.05	19.20	0.736
November	12.99	18.41	0.806	14.55	20.85	0.734	15.83	22.74	0.705
December	17.83	26.03	0.829	20.42	29.06	0.762	21.90	31.22	0.732

Table 3. Monthly precipitation amounts of the basins calculated based on the EBK (mm)

Basin	Area Km^2	Months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Meriç-Ergene	14510.7	68.6	58.1	58.2	46.3	44.1	42.9	27.6	18.5	35.9	64.5	77.1	79.8
Marmara	23113.7	90.3	72.1	68.3	53.0	41.6	38.4	24.7	28.0	44.7	79.8	92.4	108.9
Susurluk	24304.2	87.6	71.7	65.6	61.1	46.2	30.1	12.9	13.6	27.9	54.1	77.9	97.7
North Aegean	9963.6	100.3	85.9	68.3	53.3	33.9	17.7	6.2	5.7	19.6	49.9	93.3	117.1
Gediz	16981.4	93.9	80.2	65.3	55.4	39.3	19.5	7.9	7.2	16.3	42.4	74.8	101.6
Small Menderes	7027.1	125.6	103.7	77.5	51.9	31.2	10.7	3.1	2.7	14.8	45.8	94.0	132.8
Big Menderes	26017.1	95.5	78.7	64.5	51.9	39.3	20.9	10.6	8.6	14.7	41.8	71.4	99.2
West Mediterranean	21131.2	159.7	114.7	82.2	50.4	32.4	15.1	6.4	4.7	14.1	57.4	105.6	163.6
Antalya	20251.9	169.5	125.9	93.0	71.2	49.0	23.7	9.1	7.7	17.8	70.6	112.9	185.2
Burdur	6273.8	60.8	48.3	47.9	47.3	43.3	26.5	13.9	10.9	15.4	35.6	43.9	60.7
Akarçay	7954.5	48.0	41.3	45.0	50.2	49.1	33.0	14.9	12.7	16.2	38.6	40.2	53.3
Sakarya	63242.9	51.0	40.8	44.7	48.7	48.8	37.4	18.1	16.7	20.1	41.1	42.9	57.8
West Black Sea	28968.4	72.8	54.7	58.9	53.9	57.6	57.1	39.9	43.8	52.9	75.9	70.4	86.7
Yeşilırmak	39620.2	46.7	39.0	48.3	61.6	63.9	46.9	18.3	15.2	26.2	50.3	53.5	52.3
Kızılırmak	82082.5	42.4	33.7	42.3	53.6	56.4	40.4	15.5	13.9	19.8	36.2	39.5	47.4
Konya	49805.3	47.1	37.1	39.5	46.2	44.8	26.6	7.2	5.9	11.4	33.6	41.7	54.5
East Mediterranean	21751.2	118.4	79.4	67.0	46.8	35.5	15.5	5.0	4.0	10.2	49.1	84.7	128.8
Seyhan	22120.8	74.6	59.0	63.2	65.8	58.0	31.2	9.0	8.1	16.9	42.6	59.5	84.9
Asi	7904.2	119.3	105.0	99.9	70.8	50.7	18.3	6.2	7.1	30.0	65.0	87.0	125.4
Ceyhan	21482.6	100.8	83.9	88.5	77.3	58.0	23.8	7.0	5.8	17.9	51.7	75.5	105.4
Euphrates-Tigris	175882	69.9	70.1	75.7	75.3	53.6	17.8	5.4	3.7	9.0	46.4	63.0	74.5
East Black Sea	22876.1	79.5	71.8	70.7	72.7	76.2	79.4	54.9	60.0	77.8	114.2	102.6	92.2
Çoruh	20259.8	40.8	39.5	46.3	58.7	66.9	60.7	39.4	31.9	33.5	56.7	49.9	48.7
Aras	28041.2	23.4	25.6	33.8	52.2	71.6	59.3	40.1	32.0	22.8	43.1	31.7	26.0
Van	17977	40.1	44.4	55.7	69.0	59.6	25.3	11.1	7.1	12.3	49.3	52.6	45.2
Türkiye (total)	780043	70.8	60.2	60.9	60.3	52.4	32.4	15.3	13.8	21.0	50.1	62.6	77.9

Table 4. Monthly precipitation volumes of the basins calculated based on the EBK (billion m^3)

Basin	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1. Meriç-Ergene	1.00	0.84	0.84	0.67	0.64	0.62	0.40	0.27	0.52	0.94	1.12	1.16
2. Marmara	2.09	1.67	1.58	1.23	0.96	0.89	0.57	0.65	1.03	1.84	2.14	2.52
3. Susurluk	2.13	1.74	1.59	1.48	1.12	0.73	0.31	0.33	0.68	1.31	1.89	2.37
4. North Aegean	1.00	0.86	0.68	0.53	0.34	0.18	0.06	0.06	0.20	0.50	0.93	1.17
5. Gediz	1.59	1.36	1.11	0.94	0.67	0.33	0.13	0.12	0.28	0.72	1.27	1.73
6. Small Menderes	0.88	0.73	0.54	0.36	0.22	0.08	0.02	0.02	0.10	0.32	0.66	0.93
7. Big Menderes	2.48	2.05	1.68	1.35	1.02	0.54	0.28	0.22	0.38	1.09	1.86	2.58
8. West Mediterranean	3.37	2.42	1.74	1.07	0.68	0.32	0.14	0.10	0.30	1.21	2.23	3.46
9. Antalya	3.43	2.55	1.88	1.44	0.99	0.48	0.18	0.16	0.36	1.43	2.29	3.75
10. Burdur	0.38	0.30	0.30	0.30	0.27	0.17	0.09	0.07	0.10	0.22	0.28	0.38
11. Akarçay	0.38	0.33	0.36	0.40	0.39	0.26	0.12	0.10	0.13	0.31	0.32	0.42
12. Sakarya	3.23	2.58	2.83	3.08	3.09	2.37	1.14	1.06	1.27	2.60	2.71	3.66
13. West Black Sea	2.11	1.58	1.71	1.56	1.67	1.65	1.16	1.27	1.53	2.20	2.04	2.51
14. Yeşilırmak	1.85	1.55	1.91	2.44	2.53	1.86	0.73	0.60	1.04	1.99	2.12	2.07
15. Kızılırmak	3.48	2.77	3.47	4.40	4.63	3.32	1.27	1.14	1.63	2.97	3.24	3.89
16. Konya	2.35	1.85	1.97	2.30	2.23	1.32	0.36	0.29	0.57	1.67	2.08	2.71
17. East Mediterranean	2.58	1.73	1.46	1.02	0.77	0.34	0.11	0.09	0.22	1.07	1.84	2.80
18. Seyhan	1.65	1.31	1.40	1.46	1.28	0.69	0.20	0.18	0.37	0.94	1.32	1.88
19. Asi	0.94	0.83	0.79	0.56	0.40	0.14	0.05	0.06	0.24	0.51	0.69	0.99
20. Ceyhan	2.17	1.80	1.90	1.66	1.25	0.51	0.15	0.12	0.38	1.11	1.62	2.26
21. Euphrates-Tigris	12.29	12.33	13.31	13.24	9.43	3.13	0.95	0.65	1.58	8.16	11.08	13.10
22. East Black Sea	1.82	1.64	1.62	1.66	1.74	1.82	1.26	1.37	1.78	2.61	2.35	2.11
23. Çoruh	0.83	0.80	0.94	1.19	1.36	1.23	0.80	0.65	0.68	1.15	1.01	0.99
24. Aras	0.66	0.72	0.95	1.46	2.01	1.66	1.12	0.90	0.64	1.21	0.89	0.73
25. Van	0.72	0.80	1.00	1.24	1.07	0.45	0.20	0.13	0.22	0.89	0.95	0.81
Türkiye (total)	55.23	46.96	47.50	47.04	40.87	25.27	11.93	10.76	16.38	39.08	48.83	60.77

In the last step of the paper, monthly precipitation potentials of watersheds were computed on the EBK-based, which provided the best accomplishment. Firstly, the watersheds were sorted out one by one from the monthly precipitation distribution maps created for Türkiye, and monthly average areal precipitation amounts were calculated. Then, the average precipitation amounts (heights) of the watersheds were multiplied by their surface areas, and the precipitation volumes were calculated. These processes were repeated each month.

When monthly average areal rainfall amounts of Türkiye were analyzed, it was found that the highest rainfall amounts were observed in December (77.9 mm) and January (70.8 mm), and the lowest rainfall amounts were observed in August (13.8 mm), and July (15.3 mm) (Table 3). The rainfall amounts for November (62.6 mm), February (60.2 mm), March (60.9 mm), and April (60.3 mm) were very close to each other. Similarly, the rainfall amounts for May (52.4 mm) and October (50.1 mm) were also very close to each other.

Major differences were determined in monthly precipitation models and potentials of the watersheds. The watersheds with the highest rainfall amounts by month were Antalya (185.2 mm, 169.5 mm, 125.9 mm), West Mediterranean (163.6 mm, 159.7 mm, 114.7 mm), and Small Menderes (132.8 mm, 125.6 mm, 103,7 mm) in December, January, and February; Asi (99.9 mm), Antalya (93.0 mm), and Ceyhan (88.5 mm) in March; Ceyhan (77.3 mm), Euphrates-Tigris (75.3 mm) and East Black Sea (72.7 mm) in April. Between May and September, the highest rainfall amounts were observed in Northern Türkiye, particularly in the East Black Sea, followed by Aras, Çoruh, West Black Sea, and Marmara. In October, the watersheds with the most precipitation amounts were again Antalya (112.9 mm), West Mediterranean (105.6 mm), and East Black Sea (102.6 mm).

The watersheds with the lowest rainfall amounts by month were Aras in January (23.4 mm), February (25.6 mm), March (33.8 mm), November (31.7 mm), and December (26.0 mm); Konya in April (46.2 mm) and October (33.6 mm); Small Menderes in May (31.2 mm), June (10.7 mm), July (3.1 mm) and August (2.7 mm), and Tigris-Euphrates in September (9.0 mm).

Mean areal precipitation was found over 100 mm in December in nine watersheds, in January in seven, in February in four, in November in three, and in March and October in one watershed. On the other hand, the monthly average rainfall amounts of 13 watersheds in August and 11 watersheds in July were below 10 mm. In June, July, August, and September, the amount of rainfall was very little in all watersheds other than those in Northern Türkiye (Table 3; Figure 5).

Some watersheds differ from others in terms of the months in which they receive the highest rainfall amounts. These watersheds are Tigris-Euphrates (75.7 mm) in March, Van (69.0 mm) in April, Aras (71.6 mm), Çoruh (66.9 mm), Yeşilırmak (63.9 mm), and Kızılırmak (56.4 mm) in May. Among all watersheds, the greatest rainfall magnitude was in December (Antalya: 184.4 mm, Aras: 26.4 mm), and the month with the lowest magnitude was April (Ceyhan: 76.7 mm, Konya: 46.1 mm).

The months with the highest volume of rainfall in the entire of Türkiye were December (60.77 billion m^3), January (55.23 billion m^3), and November (48.83 billion m^3). The rainfall volume for February, March, and April was approximately 47 billion m^3. The total rainfall volume for the month with the lowest rainfall, August, was calculated as 10.76 billion m^3. This volume was 11.93 billion m^3 in July and 16.38 billion m^3 in September (Table 4).

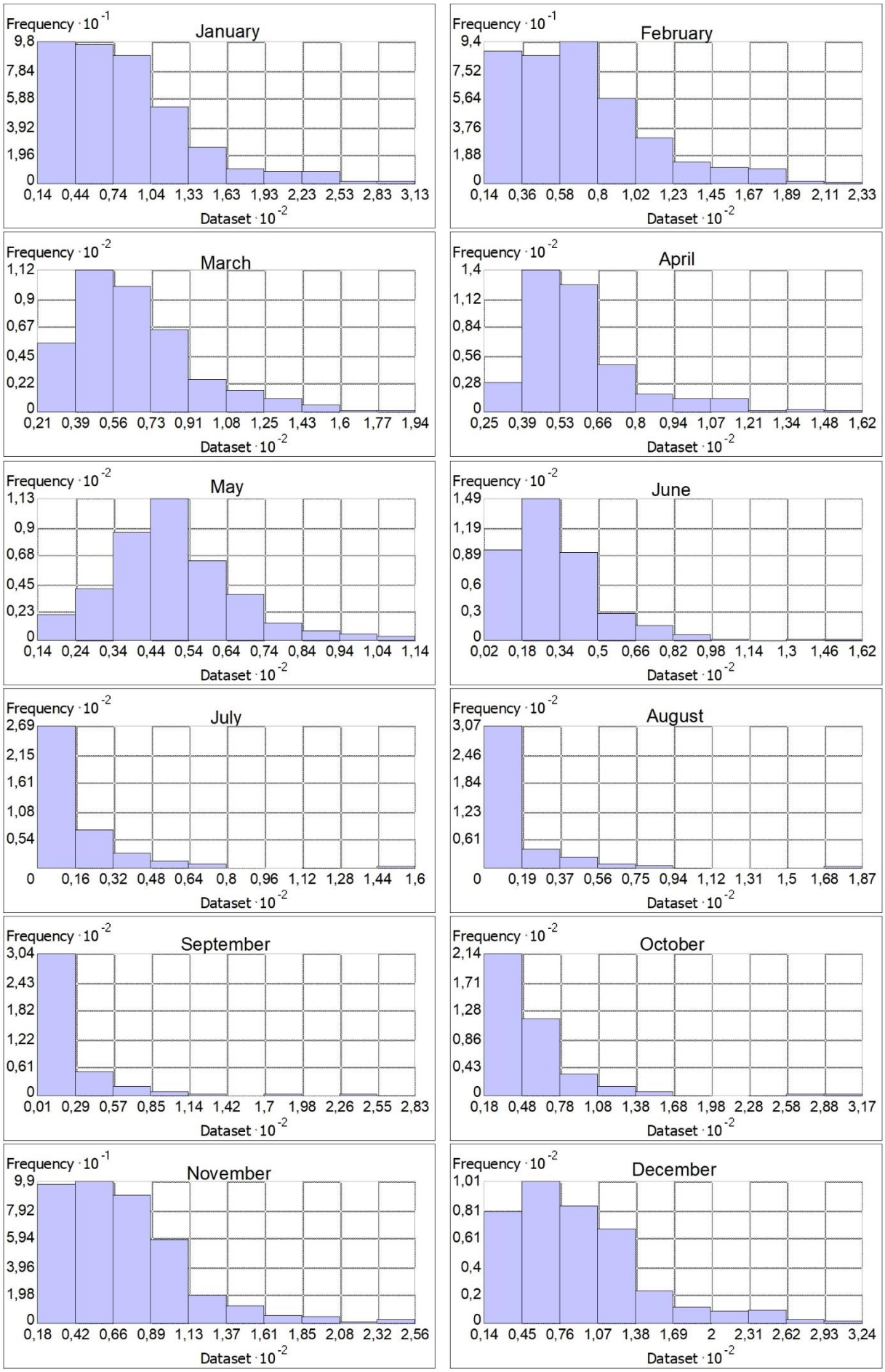

Figure 2. Monthly mean rainfall histograms of the rain gauge stations

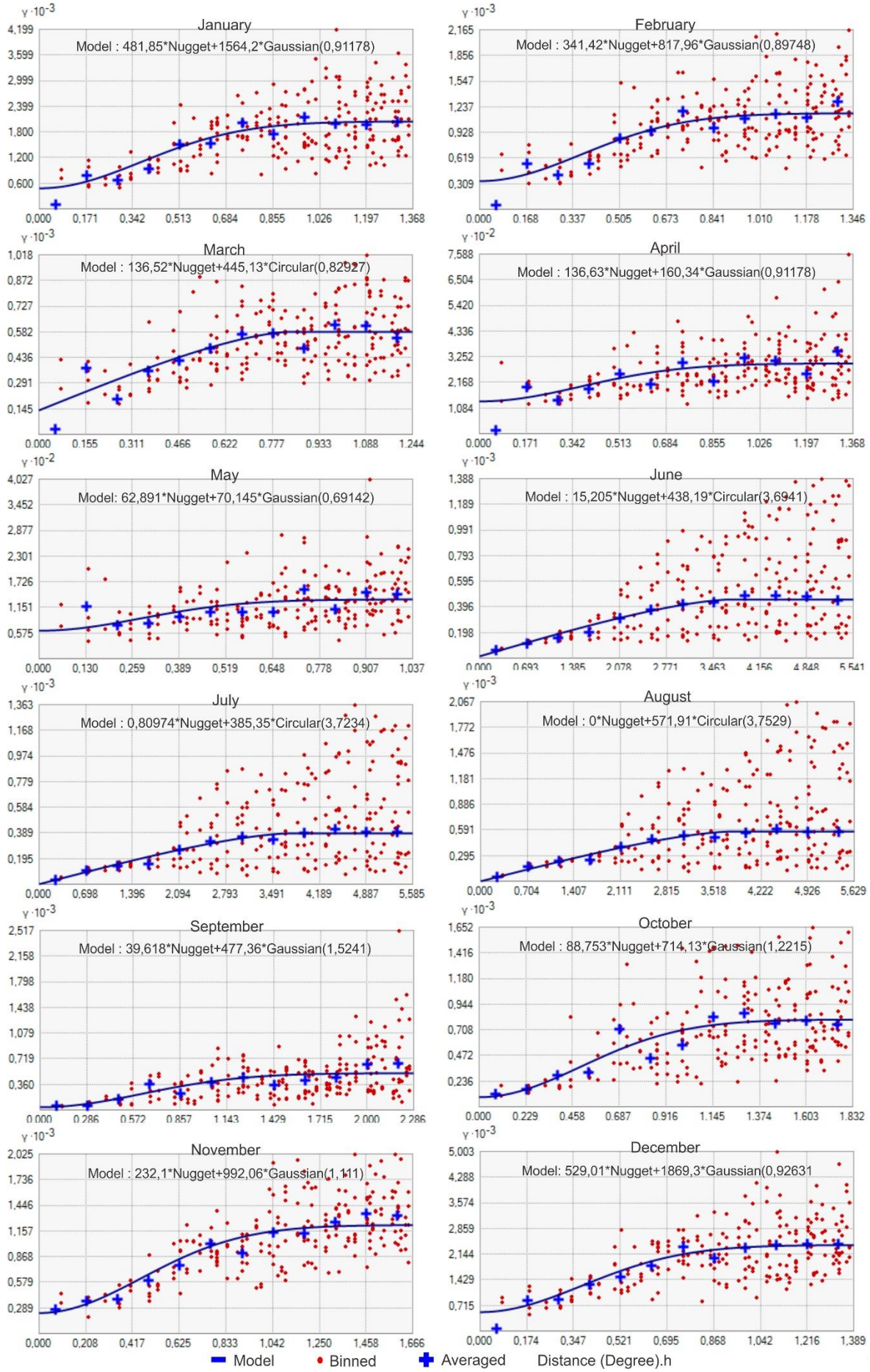

Figure 3. Models and parameters of experimental and theoretical semi-variogram for OK

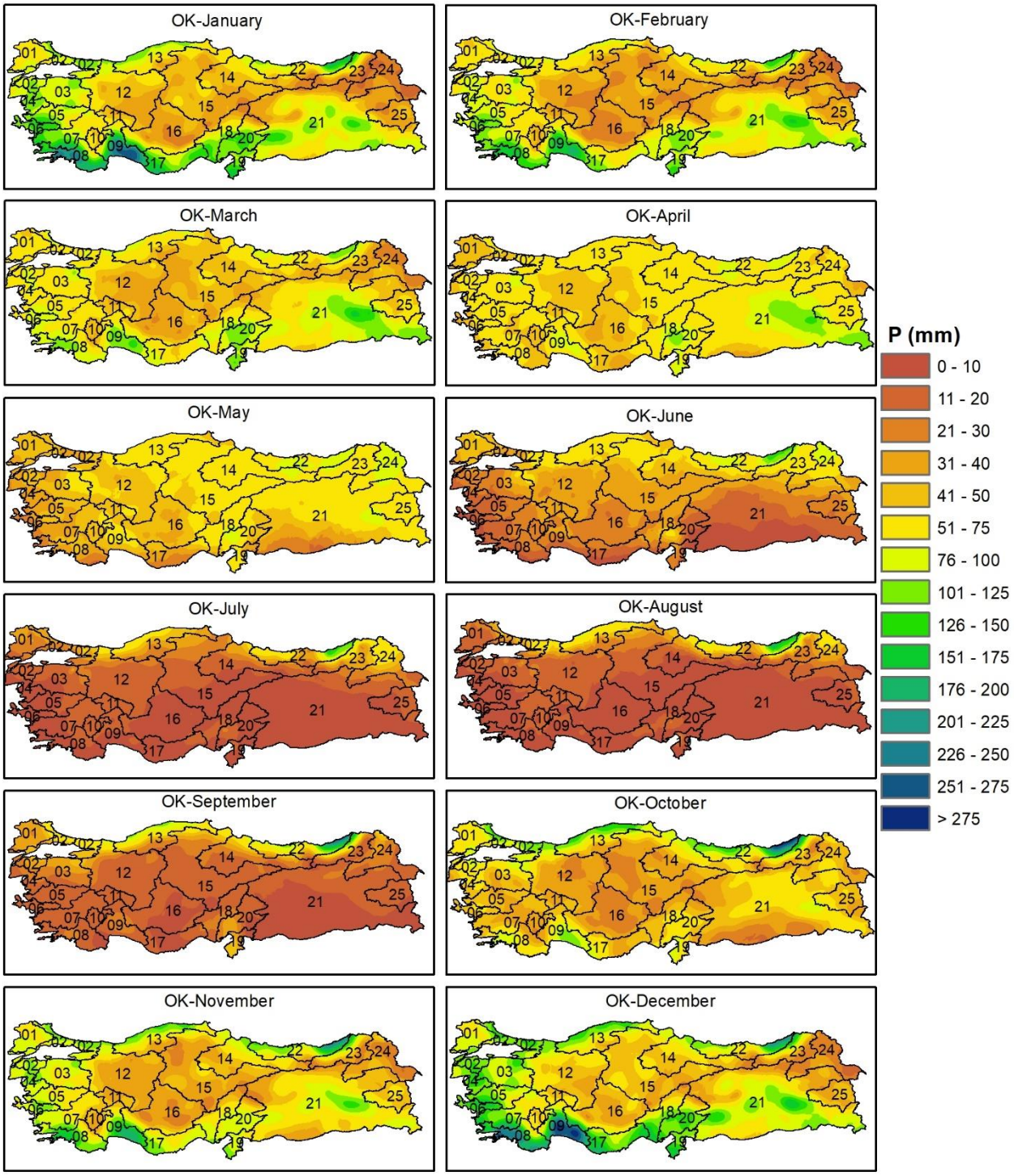

Figure 4. OK-based monthly precipitation distribution map of the basins

The watersheds with the greatest volume of rainfall were Tigris-Euphrates from October to May, Kızılırmak in June and July, and East Black Sea in August and September. The watersheds with the lowest volume of precipitation were Burdur from September to April and Small Menderes from May to August.

Discussion and Conclusions

Precipitations are considered more significant than other meteorological elements since they constitute the primary resource of fresh water, which is scarce and irreplaceable for life. In Türkiye, one of the water-scarce countries, where water resources depend on local rainfalls, it is crucial to determine the most real-like precipitation pattern and calculate the precipitation amount and volume.

The watershed-scale water administration is one of the necessary instruments for both the protection and sustainable use of water resources. Türkiye has been trying to transition to a watershed-scale water administration strategy. In the paper, the monthly precipitation distribution maps of 25 main watersheds in Türkiye were modeled, and the precipitation amounts and volumes were calculated.

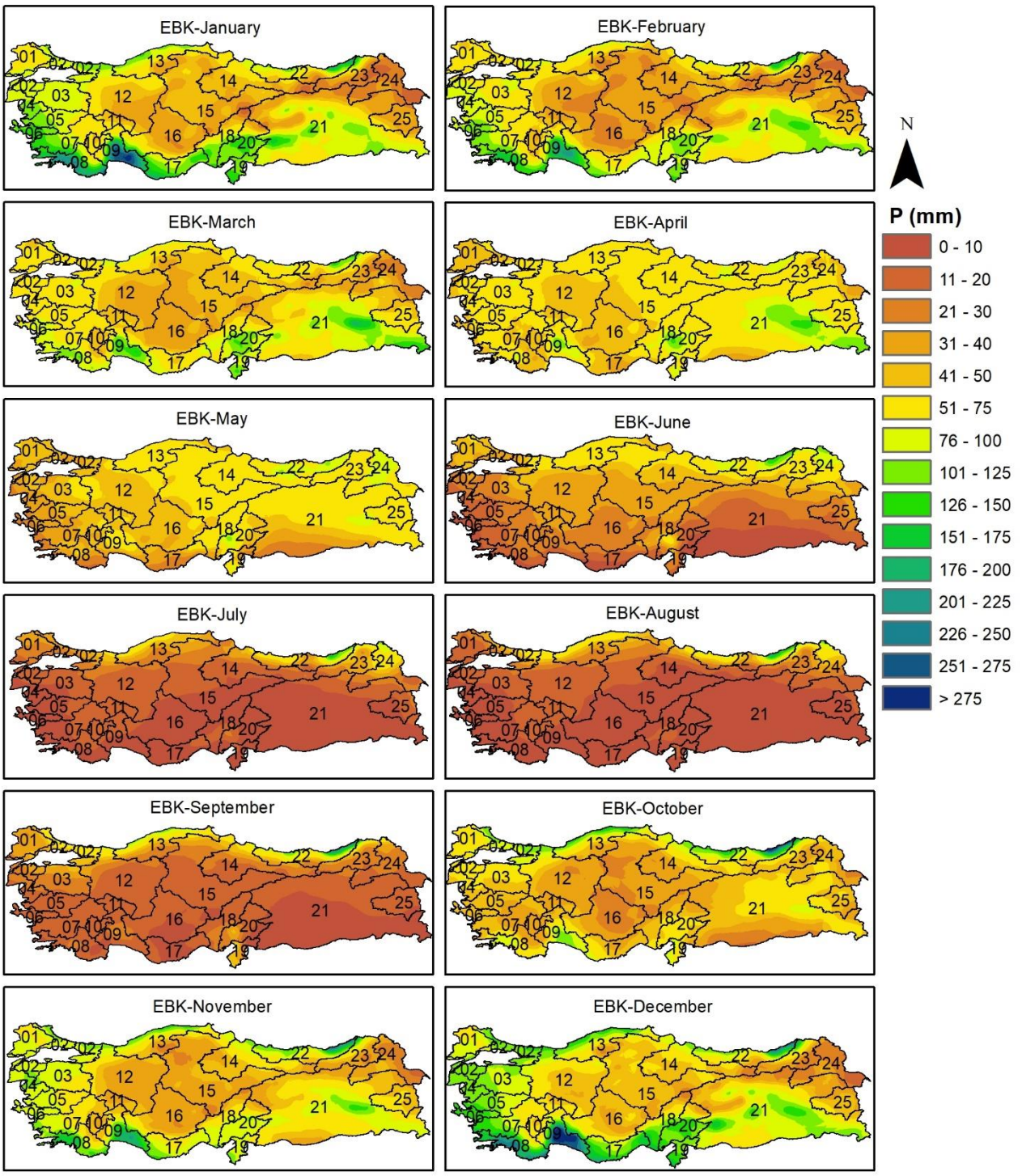

Figure 5. EBK-based monthly precipitation distribution map of the basins

The monthly precipitation data of 137 SHW raingauges, in addition to 254 SMS raingauges for the period between 1965 and 2018 were used. By combining the data of these two institutions, both the number of rain gauges required by WMO standards was achieved, and data frequency and homogeneity were ensured. Two geostatistical methods, EBK and OK, and a deterministic IDW method were used, and their performances were evaluated through cross-validation. Although all three methods showed high cross-verification performances, EBK outperformed OK and IDW in all 12 months. Similarly, OK outperformed IDW.

The months, in which the three methods showed high or low performances, were found to be parallel. RMSE and MAE amounts were low in low-precipitation months (such as July and August), and high in high-precipitation months (such as December and January). These findings are in accordance with (Pellicone et al., 2018). All three methods showed the lowest R^2 performances in April and the highest R^2 performances in July and August.

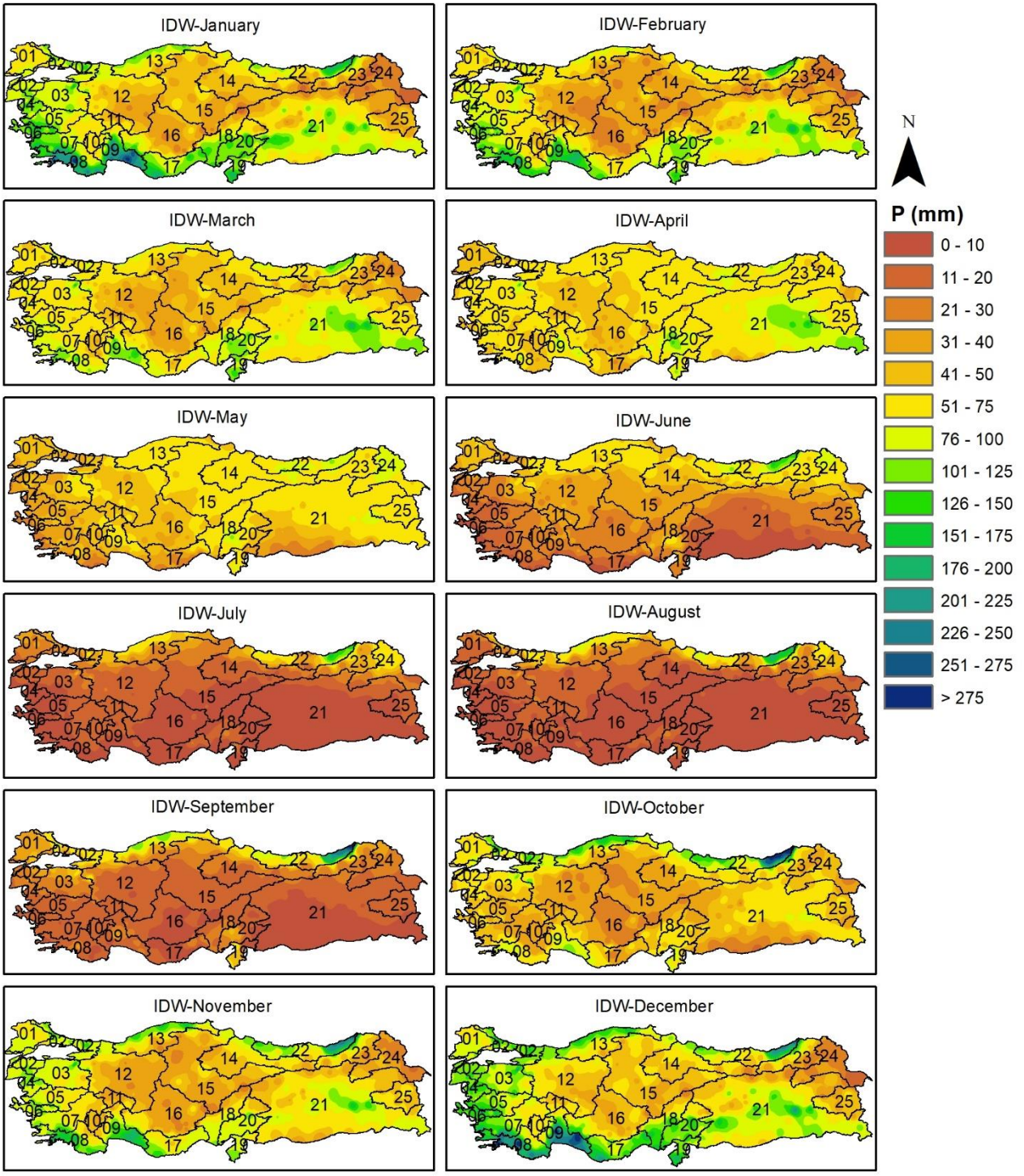

Figure 6. IDW-based monthly precipitation distribution map of the basins

Considering the high variability of the spatial distribution of monthly precipitations in Türkiye, the performances of OK and IDW methods are also adequate (Moriasi et al., 2007). The increased number of stations also increased the performance of these methods (Dirks et al., 1998; Borges et al., 2016; Frazier et al., 2016; Hurtado et al., 2021; Aksu, 2021, Aksu, 2023).

The months with the highest rainfall potential in Türkiye are December (77.9 mm, 60.77 billion m^3) and January (70.8 mm, 55.23 billion m^3) and the months with the lowest rainfall potential are August (13.8 mm, 10.76 billion m^3), July (15.3 mm, 11.93 billion m^3), and September (21.0 mm, 16.38 billion m^3). The precipitation potentials of November (62.6 mm, 39.08 billion m^3), February (60.2 mm, 46.96 billion m^3), March (60.9 mm, 47.5 billion m^3), and April (60.3 mm, 47.04 billion m^3) were almost equal. Similarly, the precipitation potentials of May (52.4 mm, 40.87 billion m^3) and October (50.1 mm, 39.08 billion m^3) were also very close to each other.

The watersheds receiving the highest rainfall amounts between November and April are located in the south and west of Anatolia and have a coast on the Mediterranean and

the Aegean Seas. Antalya in November (112.9 mm), December (185.2 mm), January (169.5 mm), and February (125.9 mm), Asi in March (99.9 mm), and Ceyhan in April (77.3 mm) are the watersheds with highest average rainfall amounts. The watershed with the highest amount of rainfall between May and October is the East Black Sea located in Northern Türkiye. On the other hand, the watersheds with the lowest rainfall amounts are Aras (between November and March), Konya (in April and October), Small Menderes (between May and August), and Tigris-Euphrates (in September).

It was determined that precipitation in Türkiye showed great differences from basin to basin within and between months. There are two main factors affecting the monthly precipitation amount and distribution of the Basins in Türkiye. The first is air masses affecting Türkiye, the second is its topographic features. Türkiye is not located in the source region of air masses. It is under the influence of weather conditions coming from different source regions according to seasons and months. Generally, it is under the influence of tropical air masses between May and October, and polar air masses between November and April (Türkeş, 2020a).

When polar air masses moving from north to south between November and April meet with tropical air masses over the Mediterranean basin, they form the Mediterranean front system (Türkeş, 2010). Cyclones, especially over the Eastern Mediterranean, bring abundant rainfall to the basins in the south and west of Türkiye, both frontal and orographically. The cyclones can penetrate the inner and eastern basins of the country, weakening by the effect of the topography. On the other hand, the air masses that come from over the Black Sea to Türkiye pick moisture and cause both frontal and orographic precipitation over the basins located in the north of the country in 12 months of the year.

Monthly precipitation amounts were found to be more regular in 12 months of the year in the East Black Sea, Aras, Çoruh, West Black Sea, and Marmara watersheds located in Northern Anatolia. The monthly rainfall amounts of the remaining 20 watersheds are extremely irregular. In the south and west of the country, the watersheds flowing into the Mediterranean and Aegean Seas, the amounts of precipitation in December exceeded 100 mm, and in August precipitation was below 10 mm.

In May, June, July, August, and September, when water, particularly agricultural irrigation, is required the most, all watersheds, except for the ones located in Northern Türkiye, are affected by the Asiatic Monsoon low-pressure system and go through a drought. These findings of the study are consistent with the previous studies Türkeş, (2010; 2020a), Hoekstra et al., (2012), and Mekonnen and Hoekstra (2016).

The use of the TSHW station data has modified the monthly rainfall models of the watersheds in Türkiye. The obtained rainfall models differ from the previous studies for Türkiye (Atalay, 2010). In the interpolation of meteorological parameters, the EBK technique should also be considered.

The pressure on the freshwater resources in Türkiye's watersheds may continue to increase in the future due to an increase in agricultural irrigation and climate change. Planning water consumption in line with the rainfall pattern and potential of the watersheds can play a key role in coping with increasing water scarcity.

The monthly precipitation potential of Türkiye is also crucial for the Middle East and its neighboring countries.

Annual evaluations of the precipitation distribution can hide the changes within the year, so it is important to prepare precipitation models monthly. For example, in Antalya and the West Mediterranean watersheds, which are among the wettest watersheds of Türkiye on an annual basis (Aksu, 2021), there is a shortage of precipitation in six months of the year.

The monthly green and blue water footprints of the Watersheds in Türkiye should be determined.

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Water-Yield Relationships of Potato in Mediterranean Climatic Conditions

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ABSTRACT

This study was carried out in Isparta University of Applied Sciences, Faculty of Agriculture, Agricultural Research, and Application Farm in 2021 to determine the effect of different irrigation water levels on tuber yield and quality parameters of the Agria potato variety. Drip irrigation method was used in the study and five different irrigation water levels (S_1: 120% of the seven-day ET_o, S_2: 90% of the seven-day ET_o, S_3: 60% of the seven-day ET_o, S_4: 30% of the seven-day ET_o, S_5: No irrigation except germination and emergence) were determined based on the reference evapotranspiration (ET_o). Irrigation water (IW) amounts varied between 85.66-639.26 mm and evapotranspiration varied between 296.54-825.15 mm. Different amounts of IW significantly affected the vegetative growth, yield and quality parameters of potato. As irrigation water decreased, total tuber yield and marketable yield declined. Total tuber yield and marketable yield were 46.11 t/ha and 40.59 t/ha, respectively, in S_1 treatment where the maximum amount of IW was applied, while they were 12.96 t/ha and 6.37 t/ha, respectively, in S_5 treatment where no irrigation was applied. Logarithmic relationships were determined between evapotranspiration and total yield and between the amount of IW and total yield. Water use efficiency was determined between 43.69-55.88 kg/(ha×mm) and irrigation water use efficiency between 32.34-51.86 kg/(ha×mm) and yield response factor (ky) was calculated as 1.19.

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Introduction

Potato (*Solanum tuberosum* L.) plant is a member of the Solanacae and has total of 200 species of which 160-180 species can produce tubers. The origin and first cultivation region of potato, which is an annual crop, is the Alpine mountains. In the late 16th century, the Spaniards brought the potato plant to their own country from the Andes mountains in the South American region. Then it spread to England, Ireland, Scotland, other European countries and other countries of the world. Asian ranks first in potato production worldwide (Berksan, 2002). According to one view, the potato was introduced to Eastern Anatolia and the Black Sea Region via Russia and the Caucasus at the end of the 19th century (Er and Uranbey, 1998; IIisulu, 1957), while according to another view, potato cultivation may have started for the first time in the Sakarya region and then spread all over Türkiye (Berksan, 2002; Er and Uranbey, 1998).

Potatoes are grown in many countries due to its tolerance in terms of climatic requirements, and they have ability to be utilized in different ways, its cheapness, high yield per unit area, high nutritional value, ease of digestion, use in human and animal nutrition, and use in industrial starch production (İncekara, 1973). Thanks to these advantages, potato ranks 7th in the world after sugar cane, maize, rice, wheat and oil palm fruit in terms of the amount of product produced (FAO, 2021). As with other plants, one of the most important cultural practices in potatoes is irrigation. Frequent irrigation application significantly affects tuber yield in potatoes (Kashyap and Panda, 2003) and the highest tuber size is obtained from treatments without deficit irrigation during the ripening period (Fabeiro et al., 2001; Yuan et al., 2003). Similarly, the increase in plant water consumption in parallel with the applied irrigation water significantly incremented tuber yield (Bahramloo and Nasseri, 2010). In addition, when irrigation water is applied together with fertilizer, which is another important cultural practice, its efficiency and water use efficiency increases (Ünlü et al., 2006). Potato is a plant that responds differently in terms of yield according to different irrigation methods, irrigation programs and irrigation method operating methods (Yavuz et al., 2012; Gültekin and Ertek, 2018; Mubarak et al.,2018).

The aim of this study was to determine the effects of water consumption, irrigation water requirement, optimum irrigation program, tuber yield and quality parameters of Agria potato variety adapted to Isparta ecological conditions under sufficient and deficit irrigation.

Material and Method

The study was conducted in Isparta University of Applied Sciences, Faculty of Agriculture, Agricultural Research and Application Farm in 2021. The Isparta province, which has an area of 8933 km^2 in the north of the Mediterranean Region, is located between 30º 20' and 31º 33' longitudes and 37º 18' and 38º 30' north latitudes, and its average height above the sea level is 1050 meters (Anonymous, 2018). The long-term average precipitation of Isparta vary between 14.1 mm and 81 mm on a monthly basis. The highest average temperature was observed in July with 23.4°C, while the lowest temperature was observed in January with 1.8°C.

Agria was used as potato variety in the study. Agria potato variety is a medium late variety with white flower color, yellow skin color and flesh color, oval and elongated tuber shape. Agria is resistant to Y virus and moderately resistant to X virus. It is generally used in the fingerling potato and chips industry (Anonymous, 2022).

Soil and Climate Characteristics of the Research Area

According to the analysis results of the soil samples (0-30 cm, 30-60 cm) of the study area, the soil texture class was determined as clay loam (CL) in both layers. The field capacity was 29.10% (130.08 mm) and 27.73% (113.14 mm), the wilting point 16.85% (75.35 mm) and 17.43% (71.10 mm), available water holding capacity was 12.25% (54.76 mm) and 10.30% (42.03 mm) in 0-30 cm and 30-60 cm soil layers, respectively (Table 1).

The total rainfall measured in 2021was 362 mm and the rainfall measured during the growing season was 134 mm. The highest average temperature was 24 °C in August and the lowest average temperature was 3.8 °C in February and December. The average relative humidity, on the other hand, was 83.6% and 36%, with the highest and lowest measured in January and August, respectively (Figure 1).

Table 1. Some characteristics of the soils in the study area

Characteristics	Unit	Soil depth, cm		
		0-30	30-60	0-60
Field capacity	%	29.10	27.73	
	mm	130.08	113.14	243.23
Wilting point	%	16.85	17.43	
	mm	75.35	71.1	146.44
Available water holding capacity	%	12.25	10.30	
	mm	54.76	42.03	96.79
Soil bulk density*	g/cm^3	1.49	1.36	
Clay	%	37.46	37.59	
Silt	%	37.08	37.21	
Sand	%	25.5	25.2	
Soil texture class		CL	CL	

* Ucar et al. (2020).

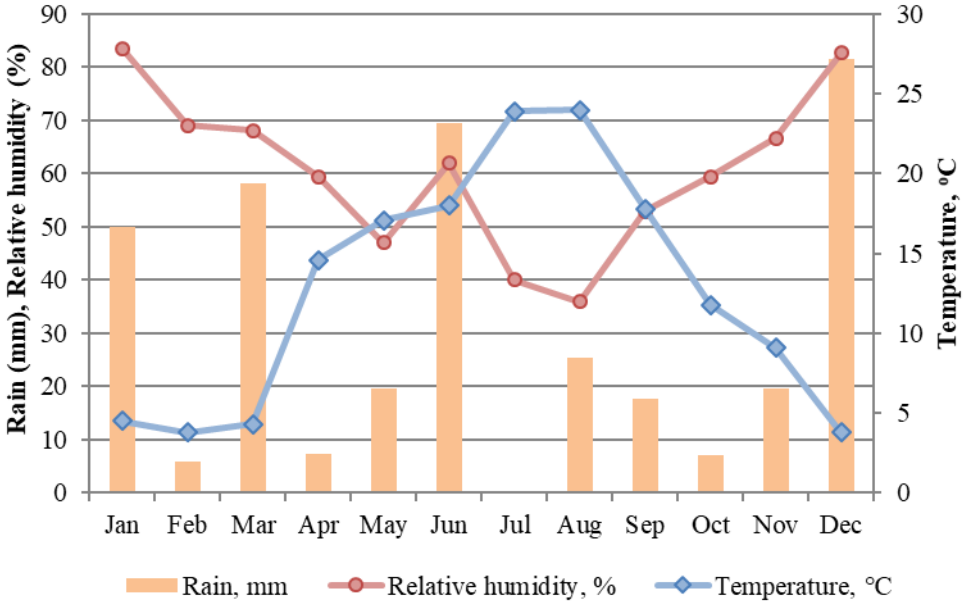

Figure 1. Some climate parameters of the trial area in 2021

Agricultural Practices

Before planting, pure 10 kg MAP (12-61-0) and 20 kg 15-15-15 compound fertilizer were applied per decare, then 30 kg Nitro Power (26% nitrogen) and 15 kg potassium nitrate (13-0-51) and potassium sulfate (0-0-51) fertilizers were applied at different stages of the development period. Insecticides were applied when potato pests were observed.

Tubers were planted on April 29 with a planting machine with 70 cm between rows and 30 cm above rows and harvested by hand on October 13. In the harvest, one row was left from each side of the plots and the remaining part was harvested.

Experimental Design

The study was carried out in three replications according to the randomized blocks design. A total of 15 parcels, each with an area of 21 m^2, were included in the experiment. The parcel length is 6 m and the parcel width is 3.5 m. Thus, the trial was carried out on a total area of 481 m^2. The irrigation interval was taken as seven days and five different irrigation water levels were created based on the reference evapotranspiration (ET_o) according to the Penman-Monteith method calculated by the meteorological station (Pessl Instruments. Metos 3.3) located 200 m away from the experiment area. Irrigation levels followed S_1: Application of 120% of the seven-day ET_o total as irrigation water, S_2: Application of 90% of the seven-day ET_o total as irrigation water, S_3: Application of 60% of the seven-day ET_o total as irrigation water, S_4: Application of 30% of the seven-day ET_o total as irrigation water, S_5: Rainfed conditions.

Irrigation Water (IW) Amount

Drip irrigation method was used in the study. The IW was applied by cumulative ET_o values calculated on daily basis, at 7-day intervals, according to the ratios specified in the trials by using equation 1. In the determination of the plant cover percentage, the plant crown with was measured before each irrigation and calculated according to equation 2 (Ertek and Kanber, 2001).

$$I = A \times Eto \times P \times R \quad (1)$$

$$P = (z/y) \times 100 \quad (2)$$

Where;

I: Irrigation water (liter), A: Plot area (m^2), ET_o: Total reference evapotranspiration (mm), P: Crop cover percentage (%), R: ET_o ratio, z: Plant crown (cm), y: Row spacing (cm)

Evapotranspiration (ET)

Effective root depth was taken as 60 cm in evapotranspiration calculations (Salimi et al., 2017). Before each irrigation, soil samples were taken from 0-30 cm, 30-60 cm soil layers. Evapotranspiration was calculated for 7-day periods using soil moisture values using equation 3 on the basis of water budget (James, 1988).

$$ET = I + P + Cp + -Dp \pm Rf \pm \Delta S \quad (3)$$

Where;

ET: Evapotranspiration (mm), I: Irrigation water amount (mm), P: Precipitation measured over a seven day period (mm), Dp: Deep percolation (mm), Cp: Capillary rise (mm), Rf: Surface runoff (mm) and ΔS: Change of water content in soil profile (mm).

Water-Yield Relationships

In determining the yield-response factor of potato, the following equation was used based on the Stewart model (Stewart et al., 1976; Doorenbos and Kassam, 1979).

$$ky = [1 - (Y/Ym)]/[1 - (ETa/ETm)] \quad (4)$$

Where;

ky: Yield response factor, Ya: Actual yield, (kg/da), Ym: Highest yield, (kg/da), ETa: Actual evapotranspiration, (mm), ETm: Maximum evapotranspiration, (mm).

Equation 5 and 6 as suggested by Howell et al., (1990) were used to calculate irrigation water use efficiency and water use efficiency.

$$IWUE = (Ey - Eyni)/I \quad (5)$$

$$WUE = Ey/ET \quad (6)$$

Where;

IWUE: Irrigation water use efficiency [kg/(ha×mm)], WUE: Water use efficiency [kg/(ha×mm)], E_y: Yield (kg/ha), E_{yni} : Yield obtained in rainfed conditions (kg/ha), I: Irrigation water (mm), ET: Evapotranspiration (mm).

Yield and Quality Parameters

Plant height (cm): In each replicate, in selected 10 plants, and their heights were measured and averaged to determine the plant height.

Number of main stem: When vegetative growth stopped, the main stems of 10 random plants were counted and averaged.

Leaf area index (LAI): Three plant samples were taken from each plot and measured with a leaf area meter and then the leaf area index was determined by proportioning the measured area to the plant crown area.

Number of tubers per plant: The number of tubers obtained from each plot after harvest was divided by the number of plants in that plot.

Tuber yield per plant (g/plant): Determined in g by dividing the total yield obtained from the harvested area by the number of plants in that area.

Average tuber weight (g): After harvesting, the average weights of the tubers in each plot were determined and the values obtained were divided by the number of tubers taken from each plant and determined as g.

Total tuber yield (t/ha): The tubers obtained from the plots were weighed and the unit area tuber yield was found by proportioning the obtained values to 1 ha surface area.

Marketable tuber yield (t/ha): The diameters of the harvested tubers were measured with the help of calipers and tubers with diameters larger than 3.5 cm were accepted as marketable tubers. The obtained tubers were weighed, and marketable tuber yield was calculated (Karadoğan, 1990).

Amorphous tuber ratio (%): It was calculated by weighing of the tubers showing amorphous development in each plot and proportioning them to the total tuber weight.

Cracked tuber ratio (%): It was calculated by weighing of the tubers showing tuber cracks in each plot and proportioning them to the total tuber weight.

Specific gravity (g/cm³): Specific bulk density of tubers was determined by applying the air-water weighing method.

Starch content (%): Calculated using the following formula (Hassanpanah et al., 2011).

$$\text{Starch (\%)} = 17{,}546 + 119{,}07 \times (\textit{Specific gravity} - 1{,}0988)$$

Dry matter ratio (%): Tuber samples were cut into thin slices and dried in an oven at 78 °C until constant weight and the dry matter weights of the tubers were determined. Dry matter ratios of the tubers were calculated by proportioning dry weights to wet weight (Şenol, 1973).

%Brix: Determined using a refractometer.

Statistical Analysis

Variance analyzes of the data obtained from the study, which was carried out in triplicate according to the randomized plot design, "IBM SPSS Statistics 23.0" made with software. If the differences between the applications were significant, Duncan's multiple comparison tests was applied.

Results and Discussion

Irrigation Water (IW) Amount and Evapotranspiration (ET)

A total of 85.66 mm of IW was applied during the 45 days after planting (DAP). After DAP 45 (when the plants reached 5-7 cm height), the irrigation programs were started. During the growing season, a total of 639.3 mm, 503.3 mm, 364.1 mm, 224.9 mm and 85.7 mm IW was applied to S_1, S_2, S_3, S_4 and S_5 treatments, respectively (Figure 2). In Bursa, Ayas and Korukçu (2010) applied 316-535 mm IW to the Hermes potato cultivar at different growth stages, while Önder et al. (2015) applied 274 mm, 182 mm, 91.5 mm, and 0 mm with surface drip irrigation method and 285 mm, 189 mm, 95 mm and 0 mm with subsurface drip irrigation method in Hatay. On the other hand, Camargo et al. (2015) stated that 796.30 mm, 694.65 mm, 581.15 mm, and 473.35 mm of IW was applied according to different irrigation subjects (60%, 80%, 100%, and 120% of plant water requirement) in the Agria potato variety. As can be understood from the previous studies, the amount of IW applied varies depending on regions where the potatoes are grown, the potato variety used, the irrigation method used in irrigation, and the irrigation program changes.

The difference in the amount of IW applied caused a difference in ET. The highest ET was measured in S_1 (825.2 mm), where 12 times the ET_o was applied, while the lowest ET was measured in S_5 (296.5 mm), where no IW was applied after the plants reached 5-7 cm. ET in S_2, S_3 and S_4 were 699.3 mm, 567.4 mm and 443.1 mm, respectively (Figure 2). According to the treatments, 74.07 mm of the measured ET was measured before the irrigation programs. Fabeiro et al. (2001) measured the maximum ET of Agria as 659 mm in Spain, while Gültekin and Ertek (2018) reported that it varied between 337.12-385.91 mm in Afyonkarahisar. Yavuz et al. (2012) determined the average ET as 670.2 mm, 618.3 mm and 572.2 mm in sprinkler, furrow, and drip irrigation methods, respectively. In another method comparison was conducted by Akram et al. (2020), ET was measured as 562 mm in furrow irrigation method and 374 mm in drip irrigation method. In Bursa, Ayaş and Korukçu (2010) reported that ET was measured between 385-651 mm under deficit water conditions applied at different growing periods. Önder et al. (2015) stated that ET between 453-714 mm in sweet potato cultivar according to the treatments, while Karataş (2018) reported it as 826.45 mm in Beniazuma sweet potato cultivar and 808 mm in Koganesengan sweet potato cultivar. As it can be seen from previous studies, ET varies according to the variety, growing region and irrigation method. It is seen that the ET of our study are slightly higher than the ET values of Agria potato in previous studies. While the long-term temperature in May, July and August in the study area were 15.5°C, 23.4°C and 23.3°C, the average temperature values in these months in the trial year were 17.1°C, 23.9°C and 24.0°C. It is thought that this increase in the average temperature values in the experimental year has an increasing effect on ET.

Tuber Yield and Quality Parameters

Statistical results showed that the IW had a similar effect on both the vegetative and generative aspects of potato. Vegetative parameters including plant height, number of main stems, and LAI statistically affected ($P<0.01$) by IW amount and varied between 48.43-70.76 cm, 2.83-4.47, and 1.00-3.03, respectively (Table 2).

Table 2. Tuber yield and quality parameters ($P<0.05$)

Parameters	F	Treatments				
		S_1	S_2	S_3	S_4	S_5
Plant height (cm)	86.626**	70.67^{a}	66.87^{b}	63.53^{c}	57.03^{d}	48.43^{e}
Number of main stems	156.679**	4.47^{a}	4.20^{b}	3.77^{c}	3.10^{d}	2.83^{e}
Leaf area index	181.526**	3.03^{a}	2.65^{b}	2.32^{c}	1.31^{d}	1.00^{e}
Number of tubers per plant	3.679*	8.04^{a}	6.72^{ab}	6.60^{ab}	6.08^{b}	5.39^{b}
Tuber yield per plant (g/plant)	87.785**	1089.3^{a}	854.28^{b}	660.82^{c}	475.50^{d}	304.27^{e}
Average tuber weight (g)	44.823**	135.71^{a}	12810^{a}	93.18^{b}	78.28^{b}	58.01^{c}
Total tuber yield (t/ha)	84.604**	46.11^{a}	36.16^{b}	26.47^{c}	20.23^{d}	12.96^{e}
Marketable tuber yield (t/da)	209.070**	40.59^{a}	30.75^{b}	21.81^{c}	14.69^{d}	6.37^{e}
Amorphous tuber ratio (%)	34.841**	2.04^{b}	2.01^{b}	1.53^{b}	2.85^{b}	18.18^{a}
Cracked tuber rate (%)	0.407^{ns}	3.7	6.07	7.59	7.12	8.66
Specific gravity (g/cm³)	23.500**	1.06^{c}	1.07^{b}	1.07^{b}	1.08^{a}	1.08^{a}
Starch rate (%)	19.712**	13.29^{d}	14.13^{c}	14.39^{bc}	14.87^{ab}	15.39^{a}
Dry matter content (%)	33.362**	17.74^{b}	19.94^{a}	20.41^{a}	20.82^{a}	21.06^{a}
Brix (%)	153.866**	4.98^{e}	5.12^{d}	5.29^{c}	5.62^{b}	5.79^{a}

*: Statistically significant at the 0.05 probability level. **: Statistically significant at the 0.01 probability level. F: F-values.

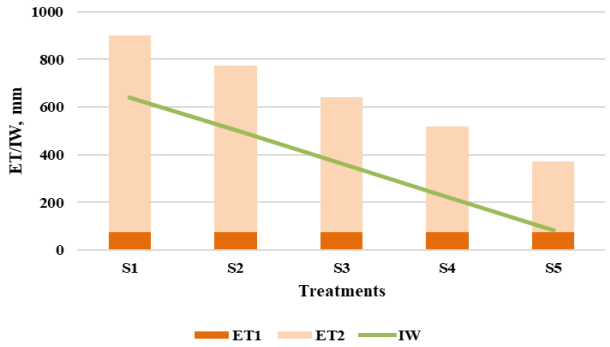

Figure 2. Irrigation water amount and evapotranspiration (ET 1: Evapotranspiration measured before irrigation programs, mm; ET 2: Evapotranspiration measured after irrigation programs, mm; IW: Irrigation water, mm)

In general, it was observed that all the vegetative parameter values decreased as the reduced irrigation water and each of them was in different statistical groups. In previous studies with different IW, Meligy et al. (2020) found plant height between 39.92 cm – 47.26 cm, Gültekin and Ertek (2018), 63.27–73.23 cm, Ayas and Korukçu (2010) 33.10-69.50 cm and Erdem et al. (2006) 86.8-98.8 cm. While Ayas and Korukçu (2010) found the number of main stems between 2.85-5.70, Gültekin and Ertek (2018) found 3.97-6.03, LAI was determined between 3.08-3.38 by Zin El- Abedin et al. (2017) and 1.4-3.92 by Salimi et al. (2017) under deficit irrigation practices.

All previous studies reported that plant height, number of main stems, and LAI were statistically affected by deficit water application (Erdem et al., 2006; Ayas and Korukçu, 2010; Zin El- Abedin et al., 2017; Salimi et al., 2017; Gültekin, and Ertek, 2018; Meligy et al., 2020). The results of plant height, number of main stems and LAI obtained from our study are consistent with some of the previous studies, while they are different from others. The differences are thought to be due to the differences in potato cultivars, growing environments, agricultural techniques, and irrigation programs.

Generative parameters including total tuber yield, marketable tuber yield, average tuber weight, tuber yield per plant and number of tubers per plant were affected ($P<0.01$) by IW and varied between 12.96–46.11 t/ha, 6.37–40.59 t/ha, 58.01–137.71 g, 304.27–1089.3 g/plant, and 5.39–8.04, respectively (Table 2).

Table 2 demonstrated that as the irrigation water increased, both total yield and marketable yield incremented, and the highest total and marketable yield were obtained from S_1 where the highest IW was applied, and all irrigation treatments were statistically different from each other in terms of total yield and marketable yield. Of the total yield, 88%, 85%, 82%, 73%, and 49% were determined as marketable yield in S_1, S_2, S_3, S_4, and S_5, respectively. Several research has been conducted to assess the effects of IW amount potato tuber yield. For example, Hassanpanah (2010) reported that the tuber yield of Agria was affected by different amounts of IW, the yield was 40.7 t/ha under full irrigation conditions, while it was 35.3 t/ha under moderate deficit water conditions and 34.6 t/ha under high deficit water conditions. In another study conducted for Agria, Eskandari et al. (2013) stated that the yield was 32.83 t/ha, 25.81 t/ha, and 19.56 t/ha under full irrigation, 30%, and 70% deficit water application compared to full irrigation, respectively, while yield was found between 45.79 kg/ha and 29.81 kg/ha by Gültekin and Ertek (2018) under Afyonkarahisar conditions. Similar results on marketable yield were found by Hassanpanah (2010), Nouri et al. (2016) and Gültekin and Ertek (2018).

The decrease in the amount of IW in potato caused a decline in the number of tubers per plant, tuber yield per plant and average tuber weight. The number of tuber per plant varied between 5.39-8.04, tuber yield per plant between 304.27–1080.30 g and average tuber weight between 58.01–135.71 g. Many researchers found similar results and stated that decreased irrigation water caused the lessened number of tubers per plant, tuber yield per plant, and average tuber weight in potato growing (Ayas and Korukçu, 2010; Badr et al., 2010; Önder et al., 2015; Nouri et al., 2016; Salimi et al., 2017; Gültekin and Ertek, 2018; Akram et al., 2020).

The effect of different irrigation water amounts on the amorphous tuber ratio was statistically significant ($P<0.01$), while the effect on the cracked tuber ratio was insignificant. Amorphous tuber ratios were found as 2.04%, 2.01%, 1.53%, 2.85%, and 18.18%, respectively, according to the treatments S_1, S_2, S_3, S_4, and S_5 (Table 2). The results correspond with Essah et al. (2020) using 'Mercury Russet' and 'Rio Grande Russet' cultivars. Although the rate of cracked tuber increases with decreasing irrigation water, the relationship between the amount of irrigation water and the rate of the cracked tuber is not clear.

Dry matter ratio and specific gravity, two important quality parameters related to tuber processing (Yuan et al., 2003), are closely related to starch content (Cantore et al., 2014). All these three quality parameters of potato were significantly affected ($P<0.01$) by the amount of IW. According to the S_1, S_2, S_3, S_4, and S_5 treatments, dry matter content was 17.74%, 19.94%, 20.41%, 20.82% and 21.06%, specific gravity was 1.06 g/cm^3, 1.07 g/cm^3, 1.07 g/cm^3, 1.08 g/cm^3, 1.08 g/cm^3 and starch content was 13.29%, 14.13%, 14.39%, 14.87% and 15.39%, respectively (Table 2). Dry matter content, specific gravity, and starch content results obtained from relatively less IW amounts applied illustrated that significantly higher than full irrigation conditions. Dry matter content, specific gravity, and starch content are affected by amounts of IW were also emphasized by many researchers such as Eskandari et al. (2013), Byrd et al. (2014). Cantore et al. (2014), Meligy et al. (2020).

The effect of the IW amount on brix was statistically significant. Brix was found as 4.98%, 5.12%, 5.29%, 5.62% and 5.79% for S_1, S_2, S_3, S_4 and S_5 subjects, respectively. As presented in Table 2, the highest brix value was determined in S_5 treatment at 5.79%, and the lowest brix value was obtained from S_1 treatment at 4.98%. Brix values decreased as the amount of irrigation water increased. All irrigation treatments were in different groups in terms of brix.

Water yield relationships

The crop yield response factor (ky) states a linear relationship between relative crop evapotranspiration and relative yield decline. It shows the yield response to relative plant evapotranspiration.

Table 3. Water use and irrigation water use efficiency

Irrigation treatment	WUE [kg/(ha×mm)]	IWUE [kg/(ha×mm)]
S_1	55.88[a]	51.86[a]
S_2	51.71[ab]	46.10[ab]
S_3	46.73[bc]	37.12[bc]
S_4	45.75[bc]	32.34[c]
S_5	43.69[c]	

(P<0.05)

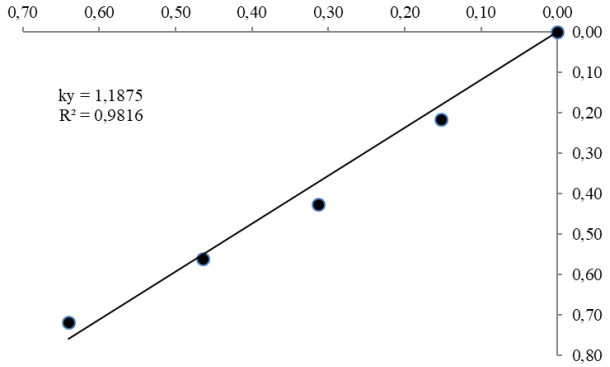

Figure 3. Yield response factor (ky)

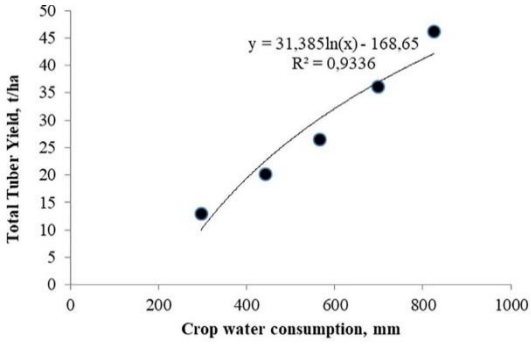

Figure 4. The relationship between evapotranspiration and total tuber yield

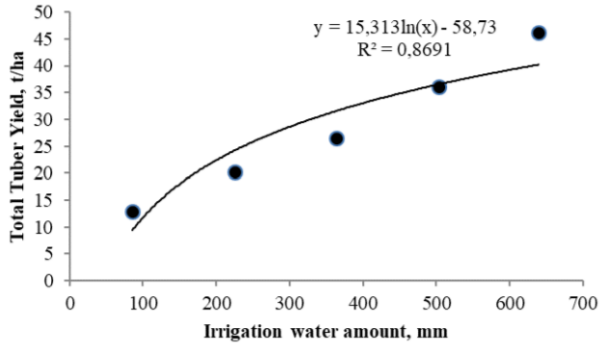

Figure 5. The relationship between irrigation water amount and total tuber yield

The yield response factor varies mainly depending on the growth stage, growth season, and severity of the water deficit (Badr et al., 2022). In other words, it accounts for yield losses due to insufficient water use (Ayas and Korukcu, 2010). The yield response factor (ky) is unique to each plant and varies according to the growing periods. A yield response factor greater than 1 indicates that the plant is sensitive to water stress and that there will be a greater decrease in yield per unit decrease in water use, while a yield response factor less than 1 indicates that the plant is more resistant to water stress and that the decrease in yield per unit decrease in water use will be lower (FAO, 2012). In this study, ky was calculated as 1.19 (Figure 3). This value shows that potato is sensitive to water stress. In the studies conducted in Türkiye on ky, Kızıloğlu et al. (2006) found ky as 1.12 under Erzurum conditions, Ayas and Korukçu (2010) found it as 0.851 under Bursa conditions, and Önder et al. (2015) found it as 1.59. On the other hand, in a study conducted for Agria under Afyonkarahisar conditions, ky was found as 2.24 (Gültekin and Ertek, 2018). The studies conducted in Türkiye differ considerably in terms of ky value. While the ky calculated in our study is similar to Kızıloğlu et al. (2006), it is remarkably different from Gültekin and Ertek (2018). This difference is thought to be due to the differences in growing conditions and agricultural techniques.

There was a logarithmic relationship between evapotranspiration and the total tuber yield with a R^2 of 0.93 [y=31.385ln(x)-168.65] and between IW and the total tuber yield with a R^2 of 0.87 [y=15.313ln(x)-58.73] (Figure 4 and Figure 5). While Kızıloğlu et al. (2006), Ayas and Korukçu (2010) and Cantore et al. (2014) reported a linear relationship between evapotranspiration and tuber yield, Aksic et al. (2014), stated that a quadratic relation between potato yield and crop evapotranspiration. Ross (2006) found a cubic polynomial relationship between potato yield and the seasonal IW amount and Karam et al. (2014) determined a strong quadratic relationship between fresh potato tuber yield and seasonal IW in Agria cultivar.

The effect of different IW amounts on WUE and IWUE is significant (P<0.05). While the lowest water use efficiency was determined for S_5 [43.69 kg/(ha×mm)] and the highest was obtained for S_1 [55.88 kg/(ha×mm)]. It was found to be 51.71 kg/(ha×mm) in the S_2 treatment, 46.73 kg/(ha×mm) in the S_3 treatment, and 45.75 kg/(ha×mm) in the S_4 treatment. The lowest irrigation water use efficiency was determined for S_5 [32.34 kg/(ha×mm)]and the highest was found for S_1 [51.86 kg/(ha×mm)]. It was determined to be 46.10 kg/(ha×mm) in the S_2 subject and 37.12 kg/(ha×mm) in the S_3 treatment (Table 3). Ayas and Korukçu (2010) reported that WUE varied between 2.99-5.23 kg/mm and IWUE varied between 1.69-4.35 kg/mm in Hermes potato variety. Akram et al. (2020) found that WUE was 5.95 kg/m^3 in furrow irrigation method and 14.1 kg/m^3 in drip irrigation method, while IWUE was 6.68 kg/m^3 in furrow irrigation and 16.3 kg/m^3 in drip irrigation. Ahuja et al. (2019) reported that WUE varied between 36.1-92.2 kg/(ha×mm) and Salih et al. (2018) reported that it varied between 59.98-99.62 kg/(ha×mm). Djaman et al. (2021) stated that potato has a high water use efficiency among the main foods (FAO, 2008), and potato WUE strongly depends upon the genetic material, management practices, irrigation regime, fertilizer rate, and other environmental conditions.

Results

In order to determine the water-yield relationships of potato, a deficit irrigation water was imposed throughout the growing season. According to the results of the study, restricted application decreased average tuber weight, tuber yield per hearth, number of tubers per hearth, total

tuber yield and marketable tuber yield. The decrease in irrigation water also negatively affected the quality parameters, and an increase in amorphous tuber and cracked tuber rates was observed with decreasing irrigation water amounts. Marketable tuber yield is the most important factor in potato cultivation in addition to other parameters. Considering the marketable tuber yield, it is predicted that S_1 can be used as an irrigation program in the study area if there is sufficient water supply, and if there are not enough water resources, S_2 can be used as an irrigation program by accepting some decreased in tuber yield.

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Comparative Fatty Acid Compositions of Tissues of Rainbow Trout (*Oncorhynchus mykiss*) with Different Ploidy and Sex

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ABSTRACT

The purpose of this study was to evaluate the fatty acid contents in various tissues (fillet, liver, gonad) of different ploidy (triploid and diploid) and sex (female and male) rainbow trout (*Oncorhynchus mykiss*) in the breeding season. In the study, diploid and triploid rainbow trouts belonging to the same age group (3+) were used. Fish were fed with commercial feed containing 45% crude protein and 20% crude fat until satiation. At the end of the 75-day study, biometric measurements of the fish were made and the tissues were stored in a deep freezer until biochemical and fatty acid analysis. The first finding of this study identified that ploidy (triploid and diploid) affects the biochemical and fatty acid composition of rainbow trout. The second major finding was that the polyunsaturated fatty acid values were higher and the saturated fatty acid values were lower in all tissues (especially female gonads) than other fatty acids. The results also indicate that the comparative among the biochemical and fatty acid composition of the fillet, liver, and gonad of rainbow trout is further illuminated by these data.

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Introduction

Fish and fish oils include omega-3 fatty acids, containging eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Holub and Holub, 2004), which originate in the food chain from phytoplankton and seaweed (Visentainer et al., 2007). Recent studies have demonstrated that the omega-3 fatty acids EPA and DHA, which are plentiful in fish tissues, have beneficial impacts on the creation of bones and metabolism as well as reducing the risk of cardiovascular disease (Su et al., 2003; Watkins et al., 2003; Lombardo et al., 2007). The total fat and fatty acid compositions of all fish species vary depending on the season, the area where they were caught, their size, diet, sex, and the stage of their reproductive cycle (Shirai et al., 2001, Shirai et al., 2002; Luzia et al., 2003; Görgün and Akpınar, 2007). While fatty fishes (such as *Salmo salar*, *Oncorhynchus mykiss*, and *Clupea harengus*) store fat primarily in muscle tissue, lean fishes store fat in body cavities and perivisceral organs (e.g. liver). The liver is a vital organ for lipid metabolism, and the majority of fish utilized for the human diet is muscle. Therefore, the livers of lean fish tend to be fatter than those of fatty fish (Uysal et al., 2006). For example, the muscles of fish used in fish oil production contain less fat than their livers (Jacobsen et al., 2022). Sexual maturation is a primary physiological process that results in a transition from somatic to gonadal development. (Taranger et al., 2010). Gonadal growth occurs at the expending of stored energy and nutrients, including fats, in many farmed fish species, including salmonids (Manor et al., 2015). Sexual maturation period, female rainbow trout grow ovaries that account for more than 20% of total body weight (Taranger et al., 2010). Because maturing females cannot digest enough nutrients from their diet to maintain gonadal growth, they must mobilize energy reserves to meet the increasing energy demand (Memiş and Gun, 2004; Salem et al., 2006, Salem et al., 2007; Aussanasuwannakul et al., 2011, Ribeiro et al., 2011; Aussanasuwannakul et al., 2012; Manor et al., 2012). Throughout maturation, lipids are mobilized from visceral adipose tissue and muscle reserves (Aussanasuwannakul et al., 2011; Manor et al., 2012). Nonetheless, the quantity and makeup of nutritional reserves, diet composition, and ratio levels are likely to influence sexual maturation and affect body composition (Manor et al., 2015). Consequently, nutrients for germinal tissue development must be obtained from other tissues such as muscle and liver. According to Uysal et al. (2006)

females primarily use saturated fatty acids to satisfy the energy requirements of gonad development, whereas males mostly use monounsaturated fatty acids. The aim of this study was to compare the fatty acid composition of muscle, liver, and gonads of rainbow trout of different sexes and ploidy.

Material and Methods

The research was carried out at Research and Application Center in Sinop University Fisheries Faculty. In the research, 9 cylindrical-shaped fiberglass tanks, each with a volume of 2000 L, were used for 75 days. The fish were divided into three groups according to sex and ploidy: diploid female (DF), diploid male (DM) and triploid female (TM). Natural flow water systems and aeration were used for each tank. Triploid and diploid rainbow trout (at 3 years old) were brought from a trading company (Kuzey Aquaculture Inc.) in Samsun, Turkey. According to the random sampling method, 9 fish with average weights >1kg (Group DF 1302.89±64.93g; Group DM 1453.55±194.03g and Group TF 1632.40±217.90g) were added to each tank from the stock tank. The Black Sea Feed (Sinop-Turkey), a commercial diet manufacturer, made the fish diet using a closed diet formula for large rainbow trout. In pursuant to the manufacturer's diet label, the biochemical composition of the diet and fatty acid composition results of diet are shown in Table 1.

Water parameters were determined daily with a field-type (YSI 556 MPS model) multiparameter measurement instrument and the water temperature was an average of 13.87±0.14ºC and the O_2 values were an average of 6.87±0.12mg/L. Fish were killed with a high dose of anesthesia (MS-222, 25–50 mg/L, Ortuno et al., 2002). The sampled fish were brought to the Faculty of Fisheries and Aquaculture. In the laboratory, fish were cut into boneless fillets by separating their internal organs and skins, they were kept in a deep freezer (WiseCryo/WUFD50080°C) until analysis. The biometric data were calculated according to Jobling (2010).The biochemical analyses of the gonad, liver and fillets samples was evaluated using AOAC-approved procedures (1990). Fatty acid analyses were performed in the Marmara Research Center of the Scientific and Technological Research Council of Turkey (TUBITAK MAM) using IUPAC gas chromatography (Firestone and Horwitz, 1979). Total fatty acids and fatty acid quality assessments were calculated according to Ulbricht and Southgate, (1991) and Santos-Silva et al., (2002). Where; AI= Atherogenicity Index; TI= Thrombogenicity Index; H/H= Hypocholesteroemic/ Hypercholesterolemic ratio

$$\mathrm{AI} = \frac{[(\mathrm{C12{:}0} + (4 \times \mathrm{C14{:}0}) + \mathrm{C16{:}0})]}{(\mathrm{MUFA} + \sum n-3 + \sum n-6)}$$

$$\mathrm{TI} = ((\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}))/[(0.5 \times \mathrm{MUFA}) + (0.5 \times \sum \mathrm{n}-6) + (3 \times \sum \mathrm{n}-3) + (\sum \mathrm{n}-3/(\sum \mathrm{n})-6)]$$

$$H/H = ((\mathrm{C18{:}1n}-9 + \mathrm{C18{:}2n}-6 + \mathrm{C18{:}3n}-3 + \mathrm{C20{:}4n}-6 + \mathrm{C20{:}5n}-3 + \mathrm{C22{:}6n}-3))/((\mathrm{C14{:}0} + \mathrm{C16{:}0}))$$

The data were presented as average values with standard error (SE). For statistical analysis, the IBM SPSS 21 statistics package program was employed. Oneway ANOVA was used to assess the importance of the data differences, and Tukey's multiple comparisons process was used to assess its accuracy.

Table 1. The biochemical and fatty acid compositions of the diets

Biochemical composition (%)*	
Crude Protein	45
Crude Fat	20
Crude Ash	10
Dry Matter	90
Fatty acid composition (%)	
C12:0	0.09±0.01
C13:0	0.01±0.01
C14:0	1.31±0.01
C15:0	0.15±0.01
C16:0	14.04±0.02
C17:0	0.20±0.01
C18:0	4.19±0.02
C20:0	0.43±0.01
C22:0	0.22±0.01
C23:0	0.01±0.01
C24:0	0.08±0.01
C14:1	0.03±0.01
C16:1	1.91±0.01
C18:1n-9c	24.17±0.01
C20:1n-9c	0.05±0.01
C24:1	0.07±0.01
C18:2n-6c	39.30±0.06
C18:3n-3	4.81±0.01
C18:3n-6	0.09±0.01
C20:2	0.18±0.01
C20:3n-3	0.06±0.01
C20:3n-6	0.05±0.01
C20:4n-6	0.18±0.01
C20:5n-3	1.21±0.02
C22:5n-3	0.18±0.01
C22:6n-3	1.55±0.01
ΣSFA	20.70±0.04
ΣMUFA	26.42±0.23
ΣPUFA	47.59±0.02

*According to the manufacturer's label, the biochemical composition of the diet

Results

The weight and length and biometric index of diploid female (DF), diploid male (DM) and triploid female (TF) are given Table 2.

Viscerosomatic index (VSI) values were in the highest TF group and lowest in the DM group, and the difference between the VSI values of female and male fish was statistically significant ($p<0.05$). Hepatosomatic index (HSI) values were like VSI values, in the highest TF group, and in the lowest DM group, the difference between the HSI values of all groups was significant ($p<0.05$).

The biochemical compositions of fish fillets and gonads are shown in Table 3. In the fillets the highest crude protein (CP) value was in the TF group, the highest crude fat (CF) value was in the DM group, and the statistical difference between the groups was significant ($p<0.05$).

Table 2. The length-weights and biometric indexes of fish

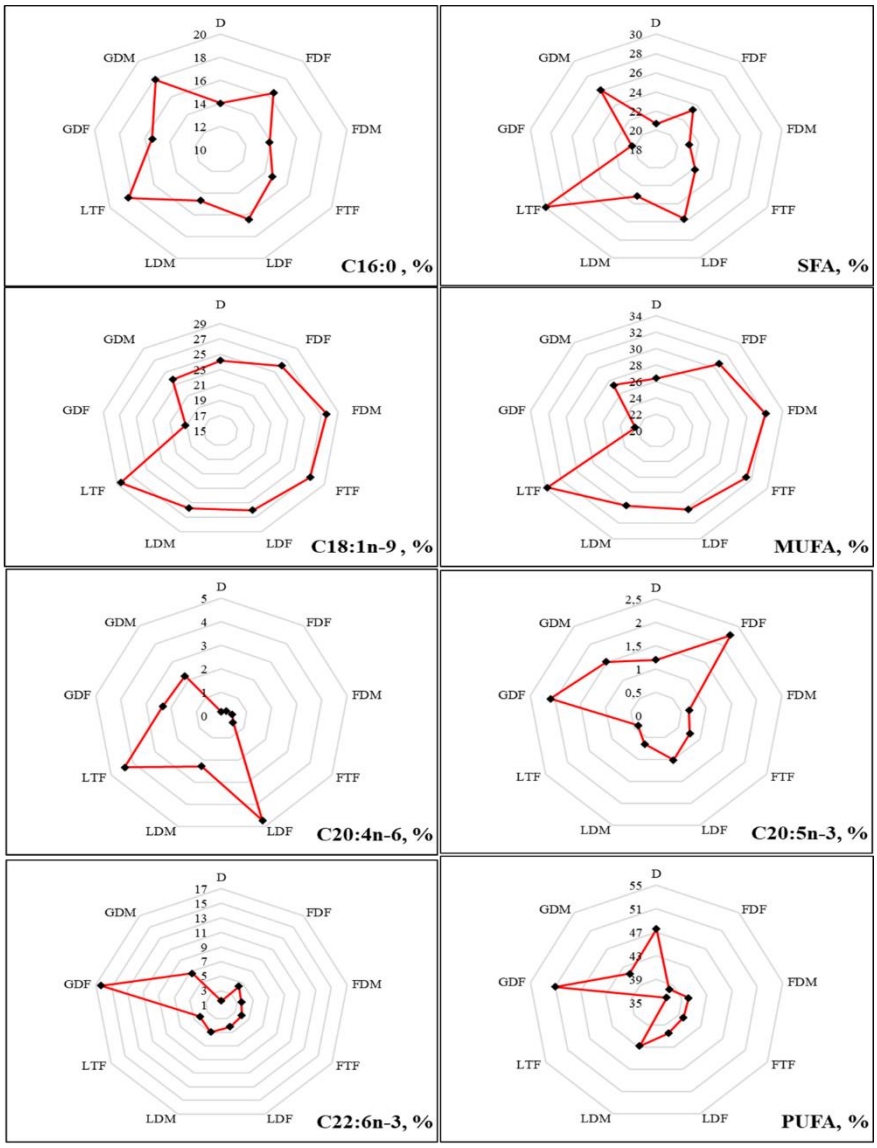
Parameters	DF	DM	TF
Weight (g)	1302.89±64.93	1453.55±194.03	1632.40±217.90
Lenght (cm)	43.24±0.66	44.58±1.34	46.40±1.39
VSI (%)	14.07±0.81^{b}	12.19±0.82^{a}	15.09±1.02^{b}
HSI (%)	1.26±0.07^{b}	1.05±0.04^{a}	1.42±0.06^{c}
GSI (%)	2.49±0.15^{b}	1.43±0.32^{a}	-

Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$); VSI= viscerosomatic index, HSI = hepatosomatic index, GSI= gonadosomatic index

Table 3. The biochemical composition of fish fillets and gonads

Biochemical composition	Fillets			Gonad	
	DF	DM	TF	DF	DM
CP (%)	21.85±0.45^{b}	20.42±0.12^{a}	22.41±0.18^{c}	21.91±0.14^{y}	20.20±0.11^{x}
CF (%)	7.14±0.24^{a}	12.19±0.03^{c}	9.10±0.08^{b}	11.83±0.15^{y}	5.86±0.66^{x}
CA (%)	1.47±0.08^{a}	1.46±0.04^{a}	1.57±0.14^{b}	2.25±0.17^{x}	2.27±0.01^{x}
DM (%)	30.05±0.64^{a}	34.23±0.28^{b}	33.21±0.28^{b}	37.87±1.21^{y}	28.89±0.47^{x}

CP= crude protein, CF= crude fat, CA=crude ash, DM= dry matter, Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$); a, b: The differences between the means with different letters on the same line within the biochemical composition of fillets are statistically significant ($p<0.05$); x, y: The differences between the means with different letters on the same line between the biochemical composition of gonads are statistically significant ($p<0.05$).



D:Diet; FDF: diploid female fillets; FDM: diploid male fillets; FTF: triploid female fillets; LDF: diploid female liver; LDM: diploid male liver; LTF: triploid female liver; GDF: diploid female gonad ; GDM: diploid male gonad

Figure 1. The selected fatty acids in feed and different tissues of rainbow trout

Table 4. Fatty acid composition of DF, DM and TF fillets (%)

Fatty acid	DF	DM	TF	Fatty acid	DF	DM	TF
C12:0	0.09±0.03^{b}	0.05±0.01^{a}	0.04±0.01^{a}	C18:3n-6	0.31±0.01^{a}	0.55±0.01^{c}	0.47±0.01^{b}
C13:0	0.01±0.01^{a}	0.01±0.01^{a}	0.01±0.01^{a}	C20:2	0.83±0.01^{a}	1.56±0.01^{c}	1.48±0.01^{b}
C14:0	2.10±0.01^{b}	1.55±0.01^{a}	1.54±0.01^{a}	C20:3n-3	0.12±0.01^{a}	0.16±0.01^{b}	0.12±0.01^{a}
C15:0	0.28±0.01^{b}	0.18±0.01^{a}	0.19±0.01^{a}	C20:3n-6	0.01±0.01^{a}	0.88±0.02^{c}	0.67±0.01^{b}
C16:0	16.43±0.01^{c}	13.89±0.01^{a}	14.68±0.03^{b}	C20:4n-6	0.29±0.01^{a}	0.43±0.01^{b}	0.53±0.01^{c}
C17:0	0.29±0.01^{b}	0.22±0.01^{a}	0.23±0.01^{a}	C20:5n-3	2.26±0.01^{c}	0.66±0.01^{a}	0.76±0.01^{b}
C18:0	3.55±0.02^{a}	4.51±0.01^{b}	4.88±0.01^{b}	C22:2	0.04±0.01^{a}	0.11±0.01^{b}	0.09±0.01^{b}
C20:0	0.38±0.02^{b}	0.33±0.01^{a}	0.31±0.01^{a}	C22:5n-3	0.41±0.01^{b}	0.35±0.01^{a}	0.41±0.01^{b}
C22:0	0.11±0.01^{a}	0.13±0.01^{a}	0.12±0.01^{a}	C22:6n-3	4.39±0.01^{a}	3.54±0.01^{a}	3.92±0.04^{b}
C23:0	0.11±0.01^{a}	0.18±0.01^{b}	0.16±0.01^{b}	ΣPUFA	38.09±0.02^{a}	40.07±0.02^{c}	39.89±0.02^{b}
C24:0	0.04±0.01^{a}	0.12±0.01^{b}	0.05±0.01^{a}	ΣOmega-3	10.47±0.01^{c}	6.66±0.06^{a}	8.09±0.04^{b}
ΣSFA	23.37±0.04^{c}	21.16±0.01^{a}	22.21±0.02^{b}	ΣOmega-6	26.75±0.03^{a}	30.85±0.01^{b}	30.24±0.01^{b}
C14:1	0.04±0.01^{b}	0.02±0.01^{a}	0.02±0.01^{a}	ΣOmega-9	27.21±0.02^{a}	29.00±0.01^{c}	28.37±0.01^{b}
C16:1	3.30±0.01^{c}	2.98±0.01^{b}	2.85±0.01^{a}	n3/n6	0.39±0.01^{b}	0.24±0.01^{a}	0.27±0.01^{a}
C18:1n-9c	26.09±0.02^{a}	27.61±0.02^{b}	27.11±0.01^{b}	n6/n3	2.55±0.02^{a}	4.08±0.02^{c}	3.74±0.02^{b}
C20:1n-9c	1.12±0.01^{a}	1.39±0.01^{c}	1.26±0.01^{b}	EPA/DHA	0.51±0.01^{b}	0.19±0.01^{a}	0.19±0.01^{a}
C24:1	0.11±0.01^{a}	0.12±0.01^{a}	0.10±0.01^{a}	EPA+DHA	6.65±0.01^{b}	4.20±0.01^{a}	4.46±0.04^{a}
ΣMUFA	30.66±0.03^{a}	32.12±0.02^{c}	31.33±0.01^{b}	AI	0.37±0.01^{b}	0.29±0.01^{a}	0.30±0.01^{a}
C18:2n-6t	26.06±0.02^{a}	29.00±0.01^{b}	28.58±0.02^{b}	TI	0.36±0.01^{a}	0.37±0.01^{a}	0.38±0.01^{a}
C18:3n-3	3.29±0.01^{b}	2.85±0.03^{a}	2.88±0.01^{a}	PUFA/SFA	1.63±0.01^{a}	1.89±0.01^{b}	1.80±0.01^{b}
				H/H	3.37±0.01^{a}	4.15±0.01^{c}	3.93±0.01^{b}

The each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$); a, b, c: The differences between the means with different letters on the same line within the group are statistically significant ($p<0.05$).

Table 5. Fatty acid composition of DF, DM and TF livers (%)

Fatty acid	DF	DM	TF	Fatty acid	DF	DM	TF
C12:0	0.21±0.02^{b}	0.12±0.01^{a}	0.36±0.02^{c}	C18:3n-6	0.50±0.01^{b}	0.42±0.01^{a}	ND
C14:0	1.39±0.01^{b}	1.42±0.01^{c}	1.20±0.01^{a}	C20:2	2.69±0.01^{b}	1.85±0.01^{a}	2.73±0.03^{b}
C16:0	16.40±0.32^{b}	14.66±0.02^{a}	18.34±0.23^{c}	C20:3n-3	ND	0.10±0.01^{a}	0.45±0.03^{b}
C17:0	ND	0.21±0.01	ND	C20:3n-6	1.71±0.04^{b}	1.22±0.01^{a}	1.67±0.03
C18:0	6.75±0.08^{b}	6.13±0.07^{a}	9.15±0.01^{c}	C20:4n-6	4.72±0.06^{b}	2.28±0.01^{a}	4.37±0.01^{b}
C20:0	ND	0.23±0.01	ND	C20:5n-3	1.01±0.09^{c}	0.65±0.01^{b}	0.41±0.05^{a}
C22:0	ND	0.15±0.01	ND	C22:5n-3	ND	0.32±0.02	ND
C23:0	0.94±0.01^{b}	0.08±0.01^{a}	0.92±0.02^{b}	C22:6n-3	4.18±0.12^{a}	4.96±0.01^{b}	4.15±0.01^{a}
ΣSFA	25.69±0.26^{b}	23.15±0.02^{a}	29.96±0.26^{c}	ΣPUFA	40.46±0.26^{b}	42.78±0.03^{c}	33.16±0.25^{a}
C16:1	2.93±0.14^{b}	2.30±0.02^{a}	2.90±0.04^{b}	ΣOmega-3	6.81±0.24^{b}	8.34±0.03^{c}	6.26±0.01^{a}
C18:1n-9c	26.00±0.04^{a}	25.76±0.03^{a}	28.55±0.08^{b}	ΣOmega-6	30.97±0.03^{b}	32.59±0.01^{c}	24.18±0.21^{a}
C20:1n-9c	1.28±0.08^{a}	1.20±0.01^{a}	1.96±0.05^{b}	ΣOmega-9	27.27±0.11^{a}	26.95±0.03^{a}	30.51±0.14^{b}
C24:1	ND	0.44±0.01^{a}	0.40±0.08^{a}	n3/n6	0.22±0.01^{a}	0.26±0.01^{b}	0.26±0.01^{b}
ΣMUFA	30.20±0.02^{b}	29.68±0.05^{a}	33.80±0.01^{c}	n6/n3	4.56±0.15^{a}	3.91±0.01^{b}	3.87±0.03^{b}
C18:2n-6t	24.04±0.08^{b}	28.68±0.03^{c}	18.14±0.18^{a}	EPA/DHA	0.24±0.01^{b}	0.13±0.01^{a}	0.10±0.02^{a}
C18:3n-3	1.63±0.03^{b}	2.32±0.02^{c}	1.26±0.09^{s}	EPA+DHA	5.18±0.20^{b}	5.60±0.02^{c}	4.56±0.07^{a}

ND:non-detected; The each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$); a, b, c: The differences between the means with different letters on the same line within the group are statistically significant ($p<0.05$).

Table 6. Fatty acid composition of DF, DM and TF gonads(%)

Fatty acid	DF	DM	Fatty acid	DF	DM
C12:0	0.02±0.01^{a}	0.09±0.01^{b}	C18:3n-3	2.34±0.01^{b}	2.13±0.01^{a}
C14:0	0.85±0.01^{a}	1.52±0.01^{b}	C18:3n-6	0.59±0.01^{b}	0.38±0.01^{a}
C15:0	0.19±0.01^{a}	0.21±0.01^{a}	C20:2	2.14±0.01^{b}	1.63±0.01^{a}
C16:0	15.44±0.01^{a}	17.92±0.02^{b}	C20:3n-3	0.05±0.01^{a}	0.08±0.01^{b}
C17:0	0.19±0.01^{a}	0.23±0.01^{b}	C20:3n-6	1.84±0.01^{b}	1.08±0.01^{a}
C18:0	3.36±0.02^{a}	5.57±0.01^{b}	C20:4n-6	2.35±0.05^{b}	2.22±0.01^{a}
C20:0	0.09±0.02^{a}	0.19±0.01^{b}	C20:5n-3	2.10±0.01^{b}	1.52±0.01^{a}
C22:0	0.01±0.01^{a}	0.12±0.01^{b}	C22:2	0.11±0.01	ND
C23:0	0.19±0.01^{b}	0.12±0.05^{a}	C22:5n-3	0.74±0.01^{b}	0.36±0.01^{a}
ΣSFA	20.32±0.01^{a}	26.13±0.09^{b}	C22:6n-3	16.34±0.05^{b}	6.71±0.04^{a}
C14:1	0.01±0.01	ND	ΣPUFA	51.07±0.01^{b}	41.49±0.11^{a}
C16:1	2.47±0.01^{b}	2.20±0.01^{a}	ΣOmega-3	21.56±0.07^{b}	10.79±0.04^{a}
C18:1n-9c	19.19±0.01^{a}	23.77±0.01^{b}	ΣOmega-6	27.26±0.07^{a}	29.08±0.06^{b}
C20:1n-9c	0.70±0.01^{a}	1.04±0.01^{b}	ΣOmega-9	19.89±0.01^{a}	24.81±0.01^{b}
C24:1	0.01±0.01^{a}	0.27±0.01^{b}	n3/n6	0.79±0.01^{b}	0.37±0.01^{a}
ΣMUFA	22.38±0.01^{a}	27.27±0.01^{b}	n6/n3	1.26±0.01^{a}	2.70±0.01^{b}
C18:2n-6t	22.49±0.02^{a}	25.41±0.05^{b}	EPA/DHA	0.13±0.01^{a}	0.23±0.01^{b}
			EPA+DHA	18.43±0.06^{b}	8.23±0.04^{a}

ND:non-detected; The each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$); a, b, c: The differences between the means with different letters on the same line within the group are statistically significant ($p<0.05$).

In the gonads, the highest dry matter (DM), CP, and CF values were determined in the DF group ($p<0.05$). The statistical difference between the crude ash (CA) values of the gonads was not significant ($p>0.05$).

The fatty acid compositions in the fillet, liver, and gonads of fish are given in Tables 4, 5 and 6, Figure 1 respectively. In the study, C12:0, C14:0, C15:0, C16:0, C17:0 and C20:0 values were high in DF, C23:0 and C24:0 values were high in DM, C18:0 value was higher in TF fillets. The highest SFA value was in the DF group and the difference among the SFA values determined in the fillets was significant ($p<0.05$). The ΣMUFA order of the fillets was as DM>TF>DF and the statistical difference among the ΣMUFA values in the fillets was significant ($p<0.05$).

The C18:3n-3, C20:5n-3, and C22:6n-3 were highest in DF fillets ($p<0.05$). While the C18:2n-6t, C18:3n-6, C20:2, C20:3n-3, and C20:3n-6 fatty acids were highest in DM fillets, the C20:4n-6 was highest in TF fillets. The ΣPUFA order of the fillets was as DM>TF>DF and the statistical difference among the ΣPUFA values in the fillets was significant ($p<0.05$).

In the study, C17:0, C20:0, C22:0, and C22:5n-3 fatty acids were not detected in the livers of both diploid and triploid female fish. In the fatty acid analysis performed in livers, ΣSFA and ΣMUFA values were higher in the TF group, and ΣPUFA values were higher in the DM group ($p<0.05$).

The EPA rankings of livers were as DF>DM>TF and the statistical difference among groups was significant ($p<0.05$). The DHA value of liver was found to be higher in both the DM group and statistically significant ($p<0.05$).

The saturated and monounsaturated fatty acids in the gonads of diploids were higher in male gonads (except C23:00 and C16:1). The polyunsaturated fatty acids were higher in diploid female gonads (except C20:3n-6). The C14:1 and C22:2 fatty acids could not be detected in diploid male gonads.

The ΣOmega-3 fatty acids of the gonads were higher in the DF group ($p<0.05$), and ΣOmega-6 and ΣOmega-9 fatty acids of the gonads were higher in the DM group ($p<0.05$).

When fatty acids in all tissues were evaluated, saturated fatty acids and monounsaturated fatty acids were determined more in fillets, liver, and gonads (Figure 1). The PUFAs such as EPA and DHA generally came to the fore in the gonads.

Discussion

There are many studies comparing growth parameters, meat yields, and biochemical compositions of diploid and triploid fish (Manor et al., 2012; Kızak et al., 2013; Weber et al., 2014; Wang et al., 2015; Karbalaei et al., 2017; Liu et al., 2018; Ignatz et al., 2020; Meiler and Kumar, 2021). The purpose of this study was to evaluate the biochemical and fatty acid compositions of differend sex and ploidy of rainbow trout's different tissues. There have been many studies comparing the biochemical composition of triploid and diploid fish, but the outcomes are different. The crude protein and ash content of TF fillets, as well as the crude fat and dry matter content of DM, were high in the current research. When the biochemical compositions of females were compared, all biochemical parameters of TF were higher than DF. Manor et al. (2014) reported that crude fat in triploid rainbow trout and crude protein in diploid rainbow trout were high. Diploid and triploid fish's biochemical characteristics showed no modification, according to Wang et al. (2015). According to Poontawee et al. (2007), ploidy had no influence on fish biochemical composition, particularly crude protein ratio. dos Santos et al. (1993) drew attention to the importance of the relationship between the biochemical composition of fish and their diet profiles and even suggested that separate dietary rations could be prepared for female and male broodstocks. Shearer (1994) and Ignatz et al. (2020) reported in their study that there is a relationship between the biochemical composition of fish, water temperature, and the protein/fat ratio of the diet. Given these findings, the fact that the TF group in our study had more crude fat and crude protein than the DF group despite feding the same diet and being raised in the same environment demonstrates the impact of ploidy. Manor et al. (2015) suggest that male and female rainbow trout may have different biochemical compositions and these differences may contribute to differences in fillet yield and quality. The finding of Manor et al. (2015) supports the difference between the biochemical composition of female and male rainbow trout in the current study.

In this study, total SFA was highest in DF and lowest in DM fillets. Many studies have found that SFA levels in diploid fish decline throughout sexual development (Manor et al., 2012; Riberio et al., 2012; Cleveland et al., 2017). The study's C16:0 and C18:0 SFAs and C18:1 and C22:1 MUFAs had the highest concentrations. Studies with Salmonid species found higher amounts of C16:0 and C18:0 from SFAs and C16:1 and C18:1 from MUFAs (Haliloğlu et al., 2004; Wang et al., 2015). Triploid female fillets had greater MUFA levels, whereas diploid female fillets have lower C18:1n-9c levels, which is consistent with the findings of do Nascimento et al. (2017). Riberio et al. (2012) revealed that MUFA and C18:1n-9c were transferred from the fillers to the gonads during this phase, while Henderson et al. (1984) noted that fish require C18:1n-9c to spawn. These literature studies explain why the C18:1n-9 and MUFA values of the diploid group fillets in this study are lower than the C18:1-9 and MUFA values of the triploid group fillets. The EPA and DHA values of DF fillets was higher than the other groups fillets. Cleveland et al. (2017) reported that contrary to our findings, the EPA value is higher in triploid fish than diploid fish and the ploidy effect in fish affects the composition of fatty acids. Similar to Ribero et al. (2012)'s findings, the n-3 PUFAs (C18:3n-6, C20:3n-3, and C20:5n-3) were higher in diploid groups particularly DHA.

The atherogenicity (AI) and thrombogenicity (TI) indices show the relationship between saturated and unsaturated fatty acids in the assessment of cardiovascular diseases (Ghaeni et al., 2013). According to Łuczynska et al. (2017), the AI and TI levels should not be greater than 1.00 for human health. The hypocholesterolemic/ hypercholesterolemic index (H/H) the fatty acid ratio based on cholesterol metabolism (Fernandes et al., 2014) and the foods with high H/H index values (>3) are believed to be better for human health. All groups in this study had AI, TI, and H/H values that were in line with those in the literature (Fernandes et al., 2014; Devadowsaon et al.,

2016; Łuczynska et al., 2017; Kaya Öztürk et al., 2019) and at levels that are safe for human health.

Even among fish tissues, there are known to be differences in fat and fatty acid composition. The main component of fish used for human nutrition is typically the muscle, and the liver plays a significant role in lipid metabolism. Therefore, learning more about the fatty acid profiles of fish living in their natural ecosystems by looking at their muscle and liver tissues can be helpful (Kiessling et al., 2001; Rodriguez et al., 2004). Under culture conditions, the fatty acids in fish tissues often reflect the fatty acids in their diet. During the reproductive period, they must mobilize their energy reserves to meet the increased energy demand during gonadal growth (Memiş and Gün, 2004; Salem et al., 2006; Salem et al., 2007; Aussanasuwannakul et al., 2011; Ribeiro et al., 2011; Aussanasuwannakul et al., 2012; Manor et al., 2012). In this study, the predominant SFA in the livers of both sexes was C16:0 (Table 5). The fatty acid present in the second-highest concentration was C18:0. TF livers had a higher amount (9.15±0.01%) of C18:0 than DF livers (6.75±0.08%). Only DM livers contained the C17:0, C20:0, and C22:0. In the liver of both sexes and ploidy, C18:1n-9 was found to be the main MUFAs. This fatty acid was higher in the fillets of DM and DF, while higher levels were found in the liver of the TF. In many studies, C18:1n-9 was determined to be the dominant MUFAs in the livers of rainbow trout, regardless of sex and ploidy (Akpinar et al., 2009; Ozório et al., 2012; Taylor et al., 2019). Although they are cultured under the same conditions and fed with the same feed, the differences in C18:1n-9 fatty acids of livers are thought to be due to ploidy and sex differences. Among the n-6PUFAs, C18:2n-6t was the main n-6PUFAs in the liver. In terms of sexes, C18:2n-6t is the highest in DM; in terms of ploidy, it was high in DF livers. Akpinar et al. (2009) reported that the most common n-6 PUFA in livers was C20:4n-6 and the highest value was in male fish livers. The differences in the literature are thought to be due to the fatty acids in fish diets.

The nutrition of broodstock is an essential factor affecting fecundity, gametogenesis, and gamete quality. Many studies have been conducted on female fish during their reproductive period, from gonad development to egg quality, from the hatching period to larval quality (Sargent et al., 2002; Perez et al., 2007; Huang et al., 2010). It is also known that the composition of fatty acids in sperm is related to the composition of fatty acids in diets, and feeding is also effective on sperm quality in male broodstocks (Pustowka et al., 2000; Jeong et al., 2002; Perez et al., 2007). It has been reported that dietary C16:0, C18:1n-9, C20:4n-6, EPA, and DHA increase the amount of gonads in male broodstocks (Perez et al., 2007). Lahnsteiner et al. (2009) reported that high amounts of C14:0, C16:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:4n-6, EPA, and DHA in rainbow trout sperm. In the current study, the mentioned fatty acids determined in sperm were high. The EPA and DHA fatty acids determined in sperm were higher than EPA and DHA values determined in diet and fillets inn the present study. This suggests that the fatty acids taken with the feed are spent on the formation of sperms.

Since their composition can affect the rate of fertilization, hatching, survival, and growth of fish larvae, fats and fatty acid research in particular have been used to evaluate egg quality (Tocher, 2010). Many fish species have demonstrated that PUFAs like C22:6n-3, C20:5n-3, and C20:4n-6 are crucial for both reproductive control and larval development (Izquierdo et al., 2001). A significant amount of long-chain n-3 PUFA, primarily C20:5n-3 and C22:6 n-3, is also present in fish eggs (Lu et al., 1979), reported that they play a positive role in preventing diseases (Lee et al., 2008). A high content of total PUFAs (51.07±0.01% of the total amount of fatty acids) was found in the fatty acid composition of DF gonads, with the contents of omega-3 (21.56±0.07%) and omega-6 (27.26±0.07%). The high sum of C18:3n-3, DHA, and EPA was primarily responsible for the higher proportion of total omega-3s in DF gonads. In the current study, the EPA+DHA value of the DF gonad was 18.43±0.06. Earlier research using the gonads of salmonid species revealed a higher value for this value (Ballestrazzi et al., 2003; Haliloğlu et al., 2003; Bekhit et al., 2009; Kalogeropoulos et al., 2012; Kowalska-Groralska et al., 2019; Murzina et al., 2019). In the present study, DHA and PUFA values of DF gonads were higher than those found in other tissues, and the SFA value was lower. In many studies, it has been emphasized that broodstocks spend SFA in gonad formation and accumulate PUFAs in their gonads (Cowey et al., 1985; Rennie et al., 2005; Cleveland et al., 2012; Mannor et al., 2014; Murray et al.,2018), and the present data are compatible with these studies.

Conclusion

In the study, a large portion of the energy received from feed was allocated to reproductive activities. While the fatty acid composition determined in the diploid and triploid trout fillets during the reproductive cycle was at similar values, significant differences were determined, especially in the fatty acid compositions of the gonads and livers. In conclusion, the comparison between the biochemical and fatty acid composition of fillets, livers, and gonads of rainbow trout with different ploidy and sex characteristics is further illuminated by these data.

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Consent to Publish

The authors agree with the study.

Author Contributions

The authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Associate Professor Dr. Birol BAKİ and Dr. Dilara KAYA ÖZTÜRK.

Declaration of interest

The authors declare that have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

All data generated or analyzed during this study are included in this published article

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Genetic Insights into Poaceae Forages: A Review of Current Marker Studies

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ABSTRACT

Forage variety development for diversified environmental conditions may benefit from the use of genomic-based breeding procedures. In today's conditions, molecular markers are used by researchers in this field to track loci and genome regions in crop breeding studies. Although earlier characterization efforts yielded useful information, morphological traits and RAPD markers have limitations when used together for genetic diversity research. Different combinations of methodologies are required for diversified aims to study different forage species at the genetic level and to connect micro level traits to macro level traits.

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Introduction

In the majority of semi-arid tropical nations, the lack of sufficient feed and fodder has been one of the main factors preventing livestock production from meeting an acceptable level (Ponnaiah et al., 2019). One of the key methods for increasing forage productivity is to assess forage crops' adaptation and performance across a range of production systems and settings (Habte et al., 2020). There is a need to introduce new, eco-friendly, and highly productive forage grass species to the market (Villegas et al., 2020). In many locations around the world, agricultural sustainability is threatened by drought and a lack of irrigation supplies. Crop variety development for these conditions may benefit from the use of genomic-based breeding procedures (Singh et al., 2022).

The benefits of combining traditional breeding methods with molecular technologies to generate fodder and turf cultivars have long been recognized by researchers and breeders. Despite this, conventional breeding is still used for the forage and grass cultivars that are currently available. This contrasts with the rise in research papers on the characterisation of germplasm resources using DNA markers and the identification of QTLs for many traits in many species (Roldán-Ruiz & Kölliker, 2010).

In crop breeding efforts, the involvement of molecular markers in research for monitoring loci and genomic areas has become standard. It is known that many of the molecular markers used in current studies are obtained from libraries of genomic DNA or randomly amplified PCR fragments. For marker-assisted breeding, mapping interested genes, and cloning genes by cloning techniques based on mapping, molecular markers are necessary (Hayashi et al., 2004). Phylogenetic study, characterisation of the germplasm, and gene introgression by backcrossing are a few further applications of molecular markers. Also, microsatellites have distinguished themselves as the preferred class of ready-to-use markers for breeding work on plants. Both RAPD (random amplification of polymorphic DNA) and restriction fragment length polymorphism (RFLP) analysis are difficult to scale to high-throughput techniques or transfer between laboratories. Amplified fragment length polymorphisms (AFLPs) and microsatellites are both effective tools for polymorphism identification. Can be evaluated at the stage of selection of molecular marker traits during several rounds of introgressions, quantify general genetic variability, calculating the percentage of a genome from a donor, identifying genes that are phenotypically

related to a particular trait under investigation, and more (Miah et al., 2013).

Poa Pratensis

Kentucky bluegrass (*Poa pratensis* L.) is regarded as a global species occupying a diversified range of various habitats because of its great adaptability, strong spreading capability, and significant expansiveness. Due to its widespread distribution and ease of adaptation to drastically diverse conditions, the species has given rise to a large variety of ecotypes that thrive in a variety of settings (Szenejko et al., 2016).

Poa pratensis is a significant temperate perennial grass species that is grown for both turf and pasture. This species can reproduce at many different and unusual ploidy levels through apomixis, giving rise to several morphologies that are genetically distinct. In the past, a variety of different Kentucky bluegrass cultivars and accessions have been identified based on typical turf performance or physical traits as well as through RAPD markers. Although earlier characterization efforts yielded useful information, morphological traits and RAPD markers have limitations when used together for genetic diversity research (Honig et al., 2010).

Molecular markers and flow cytometry are both required to distinguish between various apomictic offtypes. Sets of markers are required, and cryptic molecular variation must be taken into account for determining how similarity among hybridization progeny and cultivar differentiation may be assessed. For greater genotyping effectiveness, high-throughput genotyping platforms are essential (Bushman et al., 2018).

Total 25 SSR markers were used to genotype 247 Kentucky bluegrass varieties, experimental selections, and collections in the study by Honig et al. (2012). In addition to providing support for a revision or update of the classification system, SSR markers demonstrated a good association between genetic relatedness as determined by molecular markers and the original Kentucky bluegrass categorization system. With the existing set of SSR markers, the majority of cultivars, experimental choices, and collections could be uniquely identified. Individuals' genetic ties, as determined by SSR markers, closely matched established pedigrees.

***Brachiaria* spp.**

A highly significant forage species grown in the tropics is *Brachiaria ruziziensis*. Breeding efforts for *B. ruziziensis* may benefit from the use of genomic techniques to assist in the selection of superior genotypes. The genome of *B. ruziziensis*, however, is completely unknown. Additionally, there aren't many genomic tools, including molecular markers, available to enable *B. ruziziensis* breeding efforts (Silva et al., 2013) (Figure 1).

In contigs with a minimum of 10X coverage, Silva et al. (2013) found almost 85,000 perfect microsatellite loci. To design and synthesize the primers, the scientists have chosen only one fromcollection of 500 microsatellite loci located together with a minimum measurement of 100X. Subsequently tested a subset of 269 primer pairs, 198 of which were polymorphic, on 11 representative *B. ruziziensis* entries. Finding and generating microsatellite markers using genome-assembled Illumina single-ended DNA sequences is remarkably efficient. The markers produced for the genetic analysis and marker-assisted selection of *Brachiaria ruziziensis* can simply be put into practice. Reproductive studies are for species with unknown genomic information that could enjoy the benefits of genomic tools. This method for developing microsatellite markers is promising.

Figure 1. Formation of Brachiaria brizantha pasture in year 3 of agroforestry association with eucalyptus (de Souza et al., 2012)

In 2017, Ondabu et al. (2017) gathered 79 Brachiaria ecotypes from several locations in Kenya and investigated their genetic differences and relationships to 8 commercial variants. 22 markers identified a total of 120 distinct alleles in the 79 ecotypes. In identifying ecotypes with average diversity and polymorphism information richness, markers were quite helpful.

In order to find molecular markers for apospory, Thaikua et al. (2016) used an AFLP linkage map of the apomictic pollen donor of the first apomictic hybrid variety of brachiariagrass ('Mulato'). There were 272 markers in 29 linkage groupings on the map. In linkage group 2, twelve AFLP markers associated with apospory that were closely clustered were found. Using basic interval mapping and composite interval mapping, researchers discovered QTLs (quantitative trait loci) for leaf width, leaf width/length ratio, stem diameter, and percentage of filled seeds. The QTLs linked to significant agronomical features and AFLP markers closely linked to apospory are known to be useful for marker-assisted selection in breeding processes in brachiariagrass.

Bromus

There are approximately 150 C3 grass species in the Bromus genus, which is extensively dispersed in the temperate world. Many of these species lack taxonomical identification (Williams et al., 2011). One significant type of grass, prairie grass (*Bromus catharticus* Vahl), has the potential to be employed in systems for producing both high-quality feed and certified seed for grazing ruminants (Yi et al., 2021). A typical cool-season forage crop, *Bromus catharticus* produces a lot of biomass and grows

quickly in the winter and spring. However, because of the restricted number of genomic resources accessible, its genetic research and breeding have stagnated (Sun et al., 2021).

Based on 15 SCoT primers and 15 ISSR, Safari et al. (2019) assessed interspecifc connections in 90 accessions from 18 Bromus species. SCoT markers performed better in separating the accessions than ISSR markers. Based entirely on DNA molecular markers, the various parts of the Bromus genus split apart. Each species' accession may be distinguished using SCoT markers.

With high-throughput transcriptome sequencing use and pre-confirmed EST-SSR markers for *B. catharticus*, Sun et al. (2021) aimed to produce large amounts of genomic data. A new high-yield prairie grass strain, BCS1103, was used to harvest 11 tissue samples, including leaves, stems and seeds. 52 primer pairs with high polymorphisms were chosen out of 420 synthesized by researchers. To understand genetic diversity and population structure in twenty-four *B. catharticus* accessions from around the world. The values from the phylogenetic study are in agreement with the phenotypic clustering, which divided the investigated accessions into four clades. The Mantel analysis revealed that the genetic component generated the majority of the total phenotypic variation. Significant correlations between genetic information and plant height, stem diameter, leaf width, and biomass yield were observed. The development of a genomic library will assist in future research on *B. catharticus* and its relatives' genetics, taxonomy, and molecular breeding.

Yi et al. (2021) used a SRAP (sequence-related amplified polymorphism) marker to reveal the genetic diversity and structure of 80 fescue grass varieties (*Bromus catharticus*) from almost all over the world. From 47 SRAP primer pairs, 460 reliable bands were amplified, with 345 (75%) polymorphic bands. It was observed that five cluster separations were formed with 80 wild grass entries in PCoA and UPGMA clustering analyses, while the STRUCTURE analysis revealed that the 80 accessions had three genetic memberships.

Festuca

The Poaceae family's largest genus, Festuca, contains more than 600 species that are found worldwide in temperate grassland regions. These species are all adapted to very crowded area and various ecogeographical situations (Cheng et al., 2016). Festuca and its closely related genus Lolium are among the non-cereal grasses that have received the greatest attention from agronomists, evolutionists, and plant breeders. Hexaploid tall fescue and diploid meadow fescue are two crucial forage crops for agriculture that belong to the Festuca genus. *F. rubra* L. (Red fescue) and *F. ovina* L. (sheep fescue), which are used as forage and turf, are other fescues of some significance. Although Festuca species are substantially better resistant to abiotic conditions including drought, heat, and low temperatures, they do not compare favorably to Lolium species in terms of providing animal fodder because Festuca species exhibit poor establishment and relatively lower quality traits (Yamada, 2011).

Tall fescue is widely utilized as a turfgrass and a dominant forage grass in the pastoral and turf industries throughout temperate regions of the world. However, due to the roughness of its leaves, poor capacity for regeneration, and weak stress resistance, tall fescue's application was constrained. Modern pastoral enterprises desired new cultivars because they had greater potential than old cultivars (Lou et al., 2015).

Some of the most significant forage grasses in the entire world are part of the Festuca-Lolium species complex. A number of Festuca-Lolium complex species also exist, although these species are closely related to important cereal crops and share more than one trait with wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and other well-researched crops. It is known to have distinctive biological and genomic features. Genetic studies are problematic due to crossover structures and the scarcity of molecular markers. Genomic maps of *F. pratensis* and *L. multiflorum* were analyzed by Bartoš et al. (2011) using Diversity Array Technology (DArT) pointers and the DArtFest array. Genetic maps of *L. multiflorum* and *F. pratensis* showed that each crop contained 530 and 149 DArt markers, respectively, and 20 of these markers were reported to be mapped in both species. The ranking of the markers was done and a comparison with each other was made. A sequencing study between Brachypodium, rice, and *L. multiflorum* was conducted. A total of 96 markers were found to be significantly related to the ability to withstand freezing, and five of these markers had genetic associations with chromosomes 2, 4, and 7. Three genomic regions that were previously identified as being linked to freezing tolerance co-localized with chromosomal segments and QTLs in the population. The current research unequivocally supports the DArTFest array's potential for use in genomic analyses of the Festuca-Lolium complex.

Tall fescue and other outbreeding forage grass cultivars are generally generated by intercrossing several carefully chosen parents using the polycross method. Amini et al. (2018) assessed the use of AFLP molecular markers to maximize genetic variety in a tall fescue polycross breeding program. Two polycrosses of six parental plants with differing levels of genetic variation were developed to assess phenotypic traits and AFLP molecular markers. Six of the highest general combining ability genotypes were used to produce a fifth polycross population. The results demonstrated that parental selection with molecular marker assistance produced superior offspring, suggesting that selection based on molecular marker diversity may be a suitable strategy to enhance tall fescue's first-generation progenies.

Orchardgrass (*Dactylis glomerata* L.)

A perennial forage grass with a wide range of variation is *Dactylis glomerata* L. It is widely farmed throughout the entire temperate and subtropical growing zones of the earth (Peng et al., 2008). Long-lived, cool-season orchardgrass (*Dactylis glomerata* L.) is a fodder grass that is frequently used to make hay. Despite being economically significant, orchardgrass genome research is still in its infancy (Huang et al., 2015).

Xie et al. (2010) used cereal EST-SSRs and orchardgrass SSR markers to examine the diversity and

genetic linkages among 74 orchardgrass accessions in order to assess genetic variability and compare the degree of diversity. A total of 190 polymorphic bands were found, with an average of 6.3 alleles per SSR locus. The significant level of genetic variety was indicated by the average polymorphism rate. Additionally, research suggested that the orchardgrass diversity differentiation core may be located in northern Africa, Europe, or temperate Asia.

By using three different DNA-based methods, RAPD, Inter Simple Sequence Repeat (ISSR), and AFLP markers, Costa et al. (2016) were able to identify genetic differences between the three distinct subspecies of *Dactylis glomerata* and generate genotype fingerprints of the species. In this study, 97 bands were generated by RAPD tests, 40 of which (41.2%) were polymorphic. 54 of the 91 bands that the ISSR primers amplified (or 59.3%) displayed polymorphism. Finally, 100 bands were visible on the AFLP, 92 of which were polymorphic (92%). Researchers came to the conclusion that if the genotypes under study are closely connected. Many DNA-based techniques may be required to analyze variability. In fact, genetic variation from different sources may interact with the potential to be more or less polymorphic, or fusion may occur. The results indicated that AFLP may be the most logical molecular analysis for fingerprinting and evaluating of genetic relationships found among Dactylis glomerata genotypes.

Although orchardgrass it is a perennial feed with a high awareness plant, but rust infections have significantly decreased its quality and yield. Yan et al. (2016) evaluated genetic diversity and marker-trait relationships for rust in 75 orchardgrass accessions. By recruiting 18 EST-SSR and 21 SCOT markers. High genetic diversity was detected in the orchard. There were 164 and 289 total bands for the EST-SSR and SCoT markers, respectively. Results revealed that EST-SSRs are inferior to SCoTs in terms of marker efficiency (8.07). (4.82). Twenty band panels were connected to the rust characteristic, according to an association analysis.

Thinopyrum

Many grass species in Triticeae serve as high-value gene pools in feed and cereal crop breeding programs (Hu et al., 2012a). Many scientists choose the key wheat relative *Thinopyrum elongatum* because it has disease-resistance genes in its E genome. According to some research, the Fusarium head blight and wheat rust resistance genes can be seen on chromosome 7E of Th. Therefore, breeding molecular markers specific to chromosome 7E linked to resistance genes will play a very important mediator role in finding and applying resistant genes in Th. Additionally, it would make a significant contribution to the endeavor to develop wheat types that are disease-resistant (Chen et al., 2013) (Figure 2).

An excellent gene pool for wheat enhancement is *Thinopyrum elongatum*. Through chromosomal modification, genes for resistance to numerous biotic and abiotic stressors were introduced from *Th. elongatum* to wheat. With the help of molecular markers, breeding programs can screen a large number of genotypes for the introduction of foreign chromosomes (Hu et al., 2012b).

SLAF-seq technology has been extensively employed in molecular breeding, system evolution, and germplasm resource discovery because of its high throughput, high accuracy, and low cost. In the study by Chen et al. (2013), a total of 518 particular segments of the 7E chromosome of *Th. elongatum* were effectively amplified based on SLAF-seq. A total of 135 primers were produced according to 135 randomly selected fragments, and 89 specific molecular markers were developed for *Th. elongatum*. These markers have all been found in a range of materials, and it has been established that they are all distinct and stable. The 7E chromosome of *Th. elongatum* can be found using these markers, but they can also be utilized to provide a crucial theoretical and practical foundation for wheat breeding using marker-assisted selection (MAS).

Figure 2. Shallow rooted annual wheat (Triticum aestivum) on the right and deep rooted intermediate wheatgrass (Thinopyrum intermedium) (left). This soil profile was excavated 2.5 at meters dethp (Crews, 2016).

Guo et al. (2016) used 17 SCOT and 10 CDDP markers to investigate the evolutionary links found between these different plant families, 7 entries of Thinopyrum species, 11 entries of Triticum species, and *Hordeum vulgare*. The mean number of alleles for the markers SCoT and CDDP were found to be 8.5 and 6.6, respectively, across species. Results of CDDP markers determined that the genetic linkage was consistent between Thinopyrum spp., Triticum spp. and *H. vulgare* formed by SCOT markers. The findings concluded that Triticum species and Thinopyrum species showed the closest relationships, while *H. vulgare* was somewhat distant from both species. Seven additional markers have been added to understand the introduction of Thinopyrum chromosomes or chromosome fragments into Triticum species. Liu et al. (2018a) were able to detect alien chromatin in a wheat background using 67 Thinopyrum ponticum-specific markers and eight *Th. ponticum*-specific FISH probes designed based on SLAF-seq. By using SLAF-seq, Liu et al. (2018b) generated a physical map of the *Thinopyrum ponticum* chromosome 4Ag, located the blue-grained gene, and produced related specialized markers as well as a FISH probe.

Setaria

The grain, known as foxtail millet (*Setaria italica* L.), is of great importance in terms of food and grazing. It is grown and cultivated for consumption by human and animal populations around the world. Foxtail millet, in terms of its small genome and diploid nature, is fast emerging as a cutting-edge creation for studies of plant architecture, drought tolerance, and C4 photosynthesis in cereal bioenergy products. For this reason, studies on diversification, mapping and functional genomics in this formation should use highly polymorphic, sufficiently costly molecular markers to cover the whole genome (Zhang et al., 2014).

One of the prominent food grain crops in Asia is Setaria italica, which is grown as forage and fodder in America, Australia and Africa. Genome sequencing was received soon. Joint Genomic Institute (JGI) of the US Department of Energy and the Beijing Genomics Institute (BGI), China. Together with proso millet, foxtail millet is the second most produced millet in the world (*Panicum miliaceum*) (Gupta et al., 2012).

The most common method for using heterosis in foxtail millet is to breed male-sterile lines; however, more research needs to be done to understand the genetics of the majority of these lines. In the study of Jun et al. (2013), a highly male-sterile line, Gao146A was investigated. Genetic testing revealed that a single recessive gene was responsible for the predominantly male-sterile phenotype. One gene connected to SSR marker b234 that controls predominantly male sterility was located on chromosome VI using the F2 population obtained from the Gao146A/K103 hybrid. These findings not only helped to speed up the breeding technique known as molecular marker, but they also aided breeding by laying the groundwork for detailed mapping of this extremely male-sterile gene.

Total 28 SSR primer sets were used in the study by Kim et al. (2012) to examine the genetic diversity, population structure, and genetic interactions among 37 accessions of foxtail millet from Korea, China, and Pakistan. 37 foxtail millet accessions had a total of 298 alleles. The SSR diversity of the accession from China was higher than that of the accession from Korea or Pakistan. With a few notable exceptions, a phylogenetic tree built using the un-weighted pair group methods and arithmetic mean methodology identified three main groupings of accessions that were not consistent with geographic distribution patterns. The lack of a link between the accession clusters and their geographic locations suggests that there may have been more than one way for foxtail millet to spread from China to Korea.

Krishna et al. (2018) examined the cross-genome transferability of 26 and 101 simple sequence repeats (SSR) markers from foxtail millet and finger millet, respectively, in eight additional millets. Total cross-genome transferability of other millets was 100% for the 33 finger millet and 2 foxtail millet SSR markers. 101 finger millet SSR markers and 26 foxtail millet SSR markers, respectively, had cross-genome transferabilities ranging from 47.52% to 61.38% and from 30.76% to 69.23%. In comparison to genomic SSR (gSSR) markers, finger millet EST- SSR markers demonstrated a higher level of cross-genome transferability. Additionally, further research on genetic variation analysis, population structure, and germplasm characterisation of millets can be done using SSR markers.

Conclusion

It is highly effective to identify and generate microsatellite markers from genome-assembled Illumina single-end DNA sequences, which is encouraging for species whose breeding programs would benefit from the use of genomic tools but lack sufficient genomic knowledge. If the analyzed genotypes are closely related, more than one DNA-based approach may be needed for the investigation of variability. The breeding program, germplasm collection, and conservation will be greatly simplified as a result.

Researchers and breeders have long known that combining traditional breeding methods with molecular technologies would benefit the production of forage and turf cultivars. Despite this, conventional breeding is still used for the forage and grass cultivars that are currently available. In contrast, there are more and more research articles discussing the characterisation of germplasm resources with the help of DNA markers and the mapping of QTLs for various traits in various species. SLAF-seq technology has been extensively employed in molecular breeding, system evolution, and germplasm resource discovery due to its high throughput, high accuracy, and low cost.

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Conservation Agriculture for Sustainable Crop Productivity and Economic Return for the Smallholders of Bangladesh: A Systematic Review

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ABSTRACT

Agricultural farming is a complicated system that involves continuous interactions among its multiple components over a period of time. The series of activities involved in farming practices have enormous contributions to ensure food security for the humanity. With the passage of time, agriculture sector faces diversified challenges like high food demand of rapidly growing population, scarcity of available resources and adverse effect of climate change. In developing countries like Bangladesh, food sufficiency is mostly achieved through intensive farming which has detrimental effects on natural resources, surrounding environment, and the whole ecosystem. The review attempts to discover the potentials of conservation agriculture practices for sustainable crop productivity and economic profitability of smallholder farmers in Bangladesh. This study revealed that conservation farm management practice is a cost-effective modernized technique that has the ability to accelerate crop productivity and farmers income through minimum utilization of agricultural inputs. Although the concept of conservation agriculture is widely practiced in other parts of the world, Bangladesh is experiencing a slow rate of adoption during the last few years. The policy implication of the study suggests that the government should take coordinated and combined initiatives involving both public and private sector organizations to incorporate this concept into the mainstream agricultural system of Bangladesh.

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Introduction

Agriculture is considered as the backbone of Bangladesh supporting the livelihood of majority of the population (Ministry of Agriculture, 2021). The agriculture sectors contributed 11.6% to Gross Domestic Product (GDP) of the country (World Bank, 2021). The role of the agriculture sector to achieve economic prosperity in Bangladesh is beyond these proportionate contributions of the sub-sector to the country's GDP while 67% of the total population belongs to the countryside and a notable proportion (43%) of the total labor force is working in this sector (BBS, 2021). For attaining self-sufficiency to fulfill the food demand of the large population of Bangladesh, priority was given to increasing crop productivity through intensive farming (Akteruzzaman et al., 2012). The concept of 'Green Revolution' emerged in 1960s helps to enhance crop productivity within a short course of time but continuous use of fertilizers and pesticides, which are synthetic in nature affects soil properties reducing soil fertility (Kafiluddin & Islam, 2008). Agricultural intensification has detrimental effects on components of nature such as air, water, soil, and the population of microbes (Montgomery, 2007; Kassam et al., 2013; Dumansky et al., 2014). Traditional agricultural practices based on deep ploughing reduce soil organic matter content, and accelerate the processes of soil erosion, salinity intrusion, and leaching of soil nutrients (Mandal et al., 2020; Rani et al., 2021; Yadav et al., 2014; Pandey et al., 2015; Tomar et al., 2014; Bisht et al., 2014; Singh et al., 2013). On the other hand, the world's climate is changing rapidly due to anthropogenic activities. The extreme climatic catastrophes are also frequent and will continue to increase throughout the next few decades (IPCC, 2014). In case of Bangladesh, the rate of per capita CO_2 emission is comparatively low (0.5t/year) compared to other countries but it is one of the most victimized countries due to extreme climatic events (FAO, 2013). Adapting to the effects of climate change with the aim of fulfilling the food demand is the crucial challenge for the agriculture sector in developing countries like Bangladesh (Brouziyne et al., 2018; FAO, 2013). An increasing trend

of average temperature prevails in Bangladesh with intensified dry and wet season (Basak et al., 2013). For sustainable agricultural productivity, resources preserving conservation farming approach is the most viable solution to cope with the future challenges.

Conservation agriculture is a kind of resource preserving technique that allows least tillage practices, maintenance of soil coverage, management of crop residues and crop rotation practices with sustainable crop productivity and economic profitability (FAO, 2007). But due to the use of traditional farming systems like deep ploughing, long duration mono cropping practice, low use of organic or green manure, lack of balanced fertilization and residue management the soil quality is severely affected in most of the areas of Bangladesh (Kafiluddin & Islam, 2008). These kind of conventional farming practices resulted in reduced crop yield and economic loss. Only a small proportion of farmers (8-10%) across the globe follow conservation agriculture practices amid it assists farmers to enhance their economic profitability through minimum use of resources, low input costs and conserving natural ecosystem (Willer et al., 2008; Parrott et al., 2006; Lampkin & Padel, 1994). The conventional agricultural practices stimulate the loss of water and nutrients due to deep tillage practices and causes the emission of CO_2 contributing in global warming. On the other hand practicing conservation agriculture helps in conserving the natural ecosystem with the minimum usage of resources, eco-friendly pest management and maintain soil quality (Reicosky, 2001). Maintenance of cover crops have positive impacts on soil aggregation resulted in accumulation of soil nutrients and enhances population of beneficial microorganisms. The beneficial soil microbes can improve the capacity of water absorption, nutrient availability and soil aeration (Clapperton, 2003).

From the study of previous literature, it is found that studies conducted in Iowa State of Mexico emphasizing on maintenance of crops as a protecting cover, rotation of crops, and minimum soil destruction that helps to reduce soil erosion and nutrient loss (Mine et al., 2014). Nguema et al. (2013) conducted a study on impacts of conservation farming practices and found that conservation agriculture practices contributed to increase household income of farm families in Ecuador. A comparative study on economic, gender and labor productivity of conservation farming practices in India founded that cultivation of legumes without the application of zero or minimum soil cultivation had better economic benefit compared to conventional agriculture (Lai et al., 2012). Conservation farming practices performed better compared to organic farming and traditional farming practices to improve soil health with minimum emission of nitrogen and green-house gases noticed by Aune (2012). Akter & Gathala (2014) reported that mode of adoption of conservation practices by the farmers of Bangladesh affected by geographic location, cropping pattern and seasonal variability. Conservation agriculture practices also reported to have impacts on food security and rural livelihoods in Zimbabwe by improving crop productivity and family income (Tshuma et al., 2012). The above literature review indicated that worldwide consecutive studies were conducted on beneficial impacts of conservational agriculture but a very few of them are accomplished covering the geographic location of Bangladesh. This study would greatly contribute to understand the role of conservation farming practices for sustainable agricultural productivity and economic benefit of smallholder farming households located in Bangladesh. Therefore, the basic aim of this review study is to inspect how conservation agriculture practices contribute in achieving sustainable agricultural productivity as well as economic profitability of smallholder farming community of Bangladesh.

Materials and Methods

This research used a systematic technique of existing literature review (Tricco et al., 2018). This method is an effective and mostly preferred method to review the latest literature which allows to discover innovative knowledge in the relevant area of interest (Colicchia & Strozzi, 2012). This method of literature review brings an insight and logical diagnosis of formerly conducted research or current knowledge in the field of study to conduct the review. Moreover, a systematic literature review is more accurate and trustworthy process of literature review without any biasness (Mallett et al., 2012). This commonly used method of literature review enables the author to recognize, composite and evaluate all the available evidence or documents (qualitative or quantitative) for successful completion of the study (Van der Knaap et al., 2008; Mallett et al., 2012).

Significance of the study

Meeting the food demand of rapidly growing population becomes a major challenge for developing country like Bangladesh. Due to intensive land use and mostly mono cropping farming system soil fertility and productivity is reduced to a great extent (Akteruzzaman et al., 2012). In this case, conservation agriculture has great potentials for sustainable intensification to ensure food security and meet up of future challenges faced by agriculture sector in Bangladesh. Globally conservation agriculture becomes popular day by day but the rate is slow in developing countries like Bangladesh though it has beneficial impacts on agricultural productivity, resource conservation, low input use, climate change mitigation, and better economic return compared to conventional agriculture practices (FAO, 2016). To accomplish this review, a diverse data sources have been used including Web of Science, Science Direct, Journal Storage (JSTOR), and Scopus (Dias et al., 2019; Baier-Fuentes et al., 2019). The articles from databases were identified and used because of being peer reviewed and indexed in widely accepted scientific databases.

Searching article and inclusion

To search the articles the keywords such as conservation agriculture, agricultural or farm, productivity, sustainability, smallholders, economic benefit were used in the title of the study. Additionally the terms conventional agriculture, principles, impact, environment, prospects, challenges and Bangladesh were also used. This review was confined to the peer-reviewed articles and trusted documents of English language because of wider dissemination of useful knowledge, and information across the globe (López-Fernández et al., 2016).

The relevant research articles were identified by using the above-mentioned keywords while the other documents were excluded. To execute the review process a muti-stage screening technique was followed which involves go through the title or abstract of the study while the full or partial text of the articles were also read when it is required. A good number of articles were eliminated due to the duplication in nature. Based on the specific subject area, the irrelevant articles were eliminated while the studies those are relevant and related to conservation farming, and its potentials to enhance agricultural productivity, and aspects of economic benefit were finalized to accomplish this study.

Analysis of the articles

All the relevant and necessary information were extracted by reading the selected articles. The basic concept of conversation agriculture, comparative impact of conservation agriculture and conventional agriculture on farm productivity, environmental sustainability to mitigate climate change and economic benefit for the smallholder farmers of Bangladesh are the focal points of the study. Efforts were also given to discuss the future challenges of conservation agriculture practices to develop a revitalizing agro-ecosystem for sustainable intensification.

Findings and Discussion

Definition

Conservation farming is a cultivation technique which permits least disturbance of upper soil (zero tillage), maintenance of soil coverage, and crop alteration. It also focuses on population dynamics of soil microorganisms and triggers different naturally occurring biological process of the soil. This kind of activities result in improvement of water and nutrient use efficiency for sustainable farm productivity (Smith et al., 2016; FAO, 2016). Conservation farming is a natural ecosystem management system aims at sustainable crop productivity, economic profitability and food sufficiency by conserving the resources as well as environment (Friedrich et al., 2012). The basic pillars of conservation farming are: minimum tillage, usage of permanent soil coverage organic in nature and diversification of crops through crop rotation practice (FAO, 2016; Somasundaram et al., 2020). Conservation farming is a modernized technique that aims to ameliorate soil properties resulting in sustainable agricultural productivity and conservation of natural ecosystem (Shrestha et al., 2020; Yadav et al., 2017; Basavanneppa et al., 2017).

Historical background

The history of agriculture is ancient that has a commanding role throughout the process of civilization. The agricultural activities were started in Mesopotamia in the bank of the rivers named Euphrates and Nile since 3000 BCE (Friedrich et al., 2012; Hillel, 1998). During the primitive stage the tillage or cultivation equipment were mostly operated by human labor or animal draft power while mechanized tillage equipment became available in the nineteenth century after the industrial revolution (Friedrich et al., 2012). Deep tillage causes massive destruction of soil surface, and resulted in the emergence of idea like sowing of seeds by using seeding equipment without tillage practice (Farooq et al., 2011; Friedrich et al., 2012). Conservation farming was first introduced in the 1940s in the North America, and it took a good period of time to make this farming practice become popular among the farmers. During the 1970s, due to high price of fuel, destruction of soil motivate the farmers to adopt conservation farming practices in commercial farming (Farooq et al., 2011). During that period, the idea of zero or minimum tillage was started to practice in Brazil while zero tillage, and the idea of mulching were at the early stage of practice in West Africa (Friedrich et al., 2012; Lal, 1976; Greenland, 1975). The adoption rate of conservation farming took 20 years to reach at a satisfactory level (Farooq et al., 2011; Derpsch et al., 2010). Conservation farming gains popularity during the period of 1990s and started to spread rapidly throughout the world resulted in significant agricultural growth in Argentina, Paraguay, and southern part of Brazil (Kassam et al., 2013; Friedrich et al., 2012). The high acceptability of conservation farming practices were able to attract the attention of well renowned prospective organizations like International Fund for Agricultural Development (IFAD), Food and Agriculture Organization (FAO), World Bank, and the Consultative Group for International Agricultural Research (CGIAR) to develop resilient farming technique (Kassam et al., 2013). During 2015-2016 conservation agriculture adopted by farmers covering 12.5% of total cultivable area (180 m ha) accounted for significant increase compared to the year of 2008-2009 (Kumar et al., 2017). Presently, conservation farming technique is a prospective system of farming in North America, Brazil, Argentina and Paraguay (Kassam et al., 2013). Conservation agriculture is the most viable technique of farming as this is suitable for diversified crops in a wide range soil and climatic condition to enhance sustainable agricultural productivity.

Basic principles of conservation agriculture

Minimum soil disruption

Most of the farmers followed traditional farming system that requires tillage practices for any crop cultivation while minimum or zero tillage is required to follow conservation agriculture practices. In case of conservation agriculture, crop seeds are sown directly with minimum tillage practices after the collection of the earlier crop. Practicing of zero or minimum tillage has several beneficial impacts like it replenishes the top soil by reducing erosion caused by air and water and also saves labor cost and time. Moreover, it also conserves soil moisture by facilitating the rate of soil infiltration with improvement of soil health.

Maintenance of permanent soil coverage

Crop residues can be used to maintain permanent soil coverage. Generally, crop residues are living plant parts like leaves, stalks, straws, roots etc. kept over after harvesting of the crop. The top soil surface is covered with living mulch materials conserve soil moisture, suppress weed growth, and enhances soil fertility. Cover crops can be used as live mulch materials can be intercropped with the main crops. Use of permanent soil coverage has several advantage such as it helps to decrease soil erosion, minimizes weed growth by reducing germination and

improves nutrient use efficiency by facilitation the process of nutrient reclamation.

Crop diversification

Cultivation of same crops year after year in the same field which is termed as monocropping is the main feature of conventional agriculture causes low soil nutrient status, and organic matter content. On the other hand, conservation agriculture facilitates crop rotation and intercropping practices. Due to cultivation of crops having heterogeneous root length enhances the uptake of nutrient from different soil layer improve soil fertility and productivity. Inclusion of leguminous crops help to increase the status of nitrogen in the soil. Crop diversification also minimizes disease and pest infestation which greatly reduces the cost of production of various crops.

Conservation farming practices and their impacts on overall soil and crop management as compared to traditional practices

Conservation farming is a sustainable approach of production consists of a number of farming techniques adjusted according to the requirements of the crop species and regional climatic context. Various farming practices prevent soil depletion through conservation of natural resources with the aim of optimizing farm output. In conventional agriculture deep tillage is practiced which enhances soil loss, depletion of essential nutrients, and low content of organic substances while in case of conservation farming minimum or zero tillage is practiced to cultivate varieties of crops. Zero or minimum tillage practices maintain soil structure and triggers the beneficial processes like microbial activity, soil aeration, and the efficiency of water, and nutrient use (Bhatt, 2017; Hobbs et al., 2008). The major impacts of minimum soil disturbance includes prevention of plough pan formation, improve soil organic substance, nutrient recycling capacity, and minimum occurrence of weed. It also significantly contributes in reduction of production cost, control of green-house gas emission and minimization of air pollution through burning of crop residues and fuel (Laxmi et al., 2007). The cropping pattern of rice-wheat mostly followed zero tillage but it can also be practiced in case of sowing pulse crops like lentil, mustard, chick pea and cereals like rice and maize (CSISA, 2018). The surface seeding is a kind of conservation agriculture practice that involves broadcasting of wheat seeds on the moisture rich soil surface before or after the completion of harvest of previous crop (rice). In this practice soil structure is kept as it is and soil moisture helps in seed germination.

The traditional agriculture practice includes high labor cost, excessive weed growth and burning of crop residues while this cultivation technique is appropriate for marginal and smallholder farmers because of low labor and input cost, minimizing the weed growth and high content of organic substances (Yadav, 2019). In most of the cases, conventional agriculture method resembles plantation of crops in moisture rich soil. The use of flood irrigation system causes loss of water, formation of soil crust, excessive use of fertilizers due to leaching and run off loss and high incidence of pests and diseases (Tripathi & Das, 2017). Increasing crop productivity with limited resources (land, water), and sustainable management of environment is a major challenge of agriculture sector. In this case inclusion of bed planting method can be a viable solution that involves making of raised beds and furrows are made between the adjacent rows (Tripathi & Das, 2017). The top surface of the beds is used for seed sowing while the furrows are used for different intercultural operations like weeding, irrigation, fertilization and drainage (Tripathi & Das, 2017). Raised bed system has the advantage of desired placement of fertilizers, and management of weed by using mechanical techniques (Singh et al., 2010; Sharma et al., 2002). Bed planting system has the capacity to save 30-50% water used for irrigation compared with traditional system of plating (Singh et al., 2010; Naresh et al. 2010; Hossain, 2001). Conservation agriculture practices facilitates intercropping and diversification of crops while crop yield is boost up to 20% (Pandey et al., 2013).

Commonly practiced traditional rice cultivation system involves raising of seedlings in the nursery bed, and transplantation of 20-25 days seedlings in the rice field prepared by soil puddling. Puddled transplanting system of rice cultivation causes wastage of water through evaporation and percolation, and enhances the formation of hard plough plan that reduces soil aeration (Kaur & Singh, 2017; Farooq et al., 2011). The cultivation of transplanted rice requires high water and labor cost that drastically reduces the economic benefit of the farmers (Kaur & Singh, 2017; Pandey & Velasco, 1999). For the betterment of water and nutrient use efficiency, low labor cost and minimum emission of green-house gas an alternative cultivation technique of sowing the seeds directly in the rice field can be practiced. This technique reduces the cost of irrigation water, labor, fertilizer requirement, decreases green-house gas emission and better crop productivity (Kaur & Singh, 2017).

Precision farming is technology based farm management practice which enables collection, management and analysis of different levels of data to facilitate decision related to farm management for effective use of available resources with better productivity and profitability. Precision agriculture based on the principles of Global Navigation Satellite System (GNSS), and Global Positioning System (GPS) can be renamed as location specific crop management practices. In developing countries like Bangladesh where average farm size is 0.5ha and where 88.5% of the farmers have farm size less than 1 ha (BBS, 2018). Small and fragmented farm size causes hindrance for the development of sustainable farming system in Bangladesh. Precision agriculture is an eco-friendly and profitable farming system that ensure effective use of water, fertilizers, pesticides and other inputs (Solomon, 2020). Precision farming also help to minimize the green-house gas emission and create income generating facilities for skilled labor (Kumar, 2020). Despite having a good number of advantages the adoption scenario of precision farming is low because of involving high initial cost, requires long time to set up the system with expertise knowledge and skills, lack of infrastructural development and poor socio-economic background of farmers create obstacles for mass dissemination of this modern farming technique in Bangladesh.

Conservation agriculture for sustainable productivity and economic profitability for smallholder farmers

The economy of Bangladesh is largely depending on agriculture, as it creates employment opportunity for about

50% of the total labor force and also providing food for a population of over 160 million (BBS, 2018). The country is losing the cultivable land every year at a rate of 1% which is a major concern in upcoming days (MoA, 2021). In the process of technology adoption, an individual would will to accept any technology or practice if it has better advantages than the existing one.

Conservation agriculture has a wide range of positive impacts on agronomic management of crop farm by enriching soil organic matter content, minimizes weed growth by using crop residues or mulching, increase water and nutrient use efficiency (Saharawat et al., 2012; Jat et al., 2012; Shrestha et al., 2020), and increase the yield of diversified crops (Gathala et al., 2011). Crop residues have favorable impacts on soil amelioration, water infiltration, and control of soil loss significantly (Laxmi et al., 2007). It is evident from the findings that splash erosion can be controlled up to 85%, if 35% of the surface soil is surrounded by using organic residues or mulch materials (FAO, 2016). Conservation agriculture has sustainable economic benefit for the farmers because it is a time saving method that requires less agricultural inputs and labor requirement that can significantly reduce the production cost (Malik et al., 2005). An impact evaluation study on conservation agriculture was conducted by Uddin & Dhar (2016) found that conservation farming practices significantly increase farmers' income for the improvement of livelihood status than the non-adopter farmers in Bangldaesh. A comparative study was done by (Majumder et al., 2020) revealed that due to the adoption of conservation agriculture practices the productivity of crops including mustard, soybean, and rice were increased compared to conventional farming practices in coastal areas of Bangladesh. Conservation agriculture practices have positive impacts towards farmers' economic benefit and to improve soil quality in diversified areas of Bangladesh (Uddin et al., 2017). The low requirement of agricultural inputs and labor cost make this practice feasible and economically profitable for the smallholder farmers.

Conservation agriculture practices have better sustainability to save the environment and preserve natural ecosystem. It has several favorable impacts to improve the components of environment (soil, air and water), facilitates the process of carbon sequestration, and minimizes green-house gas emission.

Challenges involved in practicing conservation agriculture

Conservation agriculture has multiple positive impacts to improve crop productivity and economic benefit of farmers but the adoption of such kind of practices is not at satisfactory level. Conservation farming is widely practiced in Canada, Australia, United States, Argentina, and Brazil while in case of developing countries like China, India, Bangladesh and Zimbabwe the rate of adoption increased at a slow rate in the last few years (Kassam et al., 2013). The adoption of conservation farming practice is at medium level in case of Natore district of Bangladesh despite having a wide range of advantages than traditional agriculture (Poddar et al., 2017). Some major challenges create obstacles for wider dissemination and adoption of this potential farming practice at farmers' level. Most of the farmers do not have sufficient knowledge about the beneficial impacts and hands on knowledge about the location specific appropriate technology. To make the farming system productive and economically profitable, adequate knowledge on management practices of conservation agriculture is essential. Another notable constraint is poor socio-economic condition of the smallholder farmers. In case of developing and underdeveloped countries, farmers use crop residues as livestock feed or fuel purpose in most cases. The practice of burning crop residues to plant the succeeding crop in rice-wheat based cropping pattern is followed in India and neighboring countries (Laxmi et al., 2007). This kind of practice has detrimental impacts on organic matter status, and affecting the natural ecosystem. Farmers' are reluctant to adopt agricultural machinery for farming practices because of high initial cost with small and fragmented farm size make the agricultural equipment inappropriate for them. For better promotion and wider adoption of modernized technologies there should be a strong linkage between the relevant stakeholders like researchers, beneficiary farmers, extension staff and local leaders. Extension is a two-way communication between farmers and researchers. The nature and access to extension services of a country have a tremendous role for technology dissemination at farmer level (Rambhai, 1958).

Another important factor to be mentioned that, in most of the time, the outcomes of conservation farming practices are invisible during the early period of adoption (Abrol & Sangar, 2006). This also plays a crucial role for low adoption rate of this sustainable farming system.

Conclusion

The agriculture sector of Bangladesh is characterized by small fragmented land, low crop productivity, limited provision to agricultural technology and deterioration of environment. Ensuring food safety for the large sum of population, and mitigating the substantial impacts of climate change are the rising challenges in the upcoming days. In such condition, conservation farming practices have the viability to promote sustainable agricultural productivity by means of minimum use of inputs, conserve natural resources and economic benefit of the smallholder farmers. Moreover, it has the capacity to mitigate the negative effects of extreme climatic events, and environmental degradation. The policy implication suggests that the government should undertake a holistic approach for wider dissemination, and implementation of these farming practices at field level. Importance should also be given to increase knowledge, and skills of farmers through participatory training, and technology demonstrations with provision of incentives. Investments should be made to create cost effective agricultural technologies targeting the smallholder farmers of Bangladesh.

Conflict of interest

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Importance of *Pseudomonas aeruginosa* in Food Safety and Public Health

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*), the most pathogenic species among the pseudomonas species, is a bacterium that causes opportunistic infections resulting in significant damage to host tissues. *P. aeruginosa*, which is resistant to antibiotics, also causes fatal infection in human and animals. Infections caused by *P. aeruginosa* are difficult to treat due to its rapid proliferation in the environment and its ability to form biofilms that confer resistance to antibiotics. One of the main virulence factors of *P. aeruginosa* is its direct damage to host tissues, which disrupts the host's defense mechanisms. *P. aeruginosa* is a food-borne pathogen often detected in various food groups such as meat, milk, fruit, vegetables, and water. In recent years, there has been a noticeable rise in food-borne contamination with *P. aeruginosa*. New measures are urgently needed in the treatment of patients with infections due to this agent, since *P. aeruginosa* can develop resistance to most antibacterials. In this review, general information about *P. aeruginosa*, which has gained importance for public health, will be given.

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Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium that can cause various infections in human and animals (Pang et al., 2019). *P. aeruginosa* is an ubiquitous bacterium that can multiply in a wide variety of environments and foods. The most important feature that enables *P. aeruginosa* to be successful as an opportunistic pathogen is its wide metabolic diversity (Sadikot et al., 2005). Its rapid proliferation in the environment, wide range of phenotypes and genotypes, and strong virulence effect makes it important among opportunistic pathogenic microorganisms (LaBauve and Wargo, 2012). It is a well-known microorganism that causes persistent chronic infections and develops robust biofilms (Lee and Yoon, 2017).

P. aeruginosa is a pathogenic microorganism that can be found on the surface of foods such as fruits and vegetables, vegetation, water, soil and hospitals and causes many diseases (Brady, 2009). *P. aeruginosa* causes important health problems due to its presence in hospital environments. It is also considered a bacterium of great medical importance due to its adaptability to different environments and also its ability to cause chronic infections in immunocompromised individuals (Spiers et al., 2000). It can be easily recognized among other *Pseudomonas* species with its typical colony morphology, pigments and grape-like odor (Maçin, 2014). These pigments are named "*aeruginosa*" because they form a blue or greenish color at pH (Sırıken and Öz, 2017). Molecular methods are used for identification, apart from traditional methods such as sowing on media (Paul and Sinha, 2017).

The pathogenicity of *P. aeruginosa* is due to its virulence factors and antibiotic resistance in its genome (Moradali et al., 2017). These virulence factors are; lipopolysaccharides, alginate, flagella, pili, exotoxin A, pyoverdin, pyocyanin, phospholipase C, Quorum sensing and rhamnolipid (Jurado-Martín et al., 2021). *P. aeruginosa* is a common pathogen in hospitals and especially in intensive care units due to its ability to acquire resistance mechanisms against many antibiotic classes and antiseptics and to survive in humid environments (Santajit and Indrawattana 2016). *P. aeruginosa* has acquired resistance to antibiotics used to destroy environmental bacteria. In the treatment of infectious diseases, treatment of infections caused by *P. aeruginosa* is becoming increasingly difficult, especially thanks to the resistance it has developed against multiple antibiotic classes (Jeukens et al., 2019). Therefore, it is necessary to develop new antimicrobials to prevent the antibiotic-resistant pathogen *P. aeruginosa* (Xu et al., 2019). This review will focus on *P. aeruginosa*, which has gained importance in terms of public health.

Classification

Pseudomonadaceae family consists of 5 genera: *Azotobacter, Mesophilobacter, Oblitimonas, Permianibacter* and *Pseudomonas*. The genus *Pseudomonas* also includes different subspecies. These subspecies are classified according to the 16s ribosomal nucleic acid (rRNA) gene sequence and pigments. These; *P. aeruginosa, P. alcaligenes, P. fluorescens, P. fragi, P. mendocina, P. oleovorans, P. pseudoalcaligenes, P. putida, P. stutzeri, P. pseudomallei, P. mallei, P. solanacearum, P. marginalis, P cepacia* and *P. syringae* (Charles et al., 2006). The most important animal pathogens are *P. aeruginosa, P. pseudomallei* and *P. mallei*. The most important plant pathogens are *P. solanacearum, P. syringae* and *P. marginalis* (Bilgehan, 2000). It was defined by Schroeter in 1872 with the special name *Aeruginosa* because the color of the colonies in certain environments turns to coppery rust or copper color and then turns green. Schroeter added it to the genus *Bacterium* and named it *Bacterium aeruginosum*. Later, Migula redefined the species and transferred it to the genus *Pseudomonas*. The scientific classification made by Migula is shown in Table 1. (Migula, 1894; Palleroni, 2010).

Table 1. Scientific classification of Pseudomonas and some species (Migula, 1894).

Scientific Classification		Some Species
Domain	Bacteria	*P. aeruginosa*
Phylum	Proteobacteria	*P. gelidicola*
Class	Gamma proteobacteria	*P. fluorescens*
Order	Pseudomonadales	*P. fragi*
Family	*Pseudomonadaceae*	*P. putida*
Genus	*Pseudomonas*	*P. syringea*

Morphology and General Characteristics of Pseudomonas aeruginosa

P. aeruginosa has a Gram-negative, motile, non-spore forming, rod-shaped, monoflagella structure. *P. aeruginosa*, which is positive for catalase, oxidase, and citrate in biochemical tests, cannot ferment glucose and lactose. It is easily detectable on agar because it produces water-soluble pigments such as pyoverdin, a yellow-green fluorescent pigment, and pyocyanin, a blue-green pigment (Peix et al., 2019). These pigments are called "*aeruginosa*" because they form a blue or green-like color when the pH becomes alkaline (King and Philips, 1978).

Pseudomonas spp. it is a bacterium that can grow in aerobic and humid environments. Unlike other species in this genus, *P. aeruginosa* can also live in anaerobic conditions due to its ability to use nitrate (NO_3). In addition, they are known as an opportunistic pathogen that causes significant problems in the food industry, pharmaceutical industry and hospital environments due to their resistance to wide temperature ranges (20-42°C), high salt concentrations, many antibiotics and environmental conditions for their multiplication, as well as being able to reproduce even in distilled water. (Sırıken and Öz, 2017; Özdemir et al., 2009). Optimal growing temperature is 37°C and it has been observed that they can reproduce between 20°C and 42°C. In addition, their ability to grow at 42°C makes it easy to distinguish this bacterium from many other *Pseudomonas* species (Wu et al., 2015). They can provide the minimum nutritional conditions necessary for the reproduction of *Pseudomonas* by using a wide variety of environmental resources. *P. aeruginosa* generally requires only acetic acid salt acetate (CH3COO) and ammonia as a carbon and nitrogen source (Abreu et al., 2014).

Virulance Factors of *Pseudomonas*

P. aeruginosa, being an opportunistic pathogen, has the ability to cause both acute and chronic infections. Its pathogenic profile is due to the large and variable virulence factors and antibiotic resistance markers that reside in the genome of *P. aeruginosa* (Moradali et al., 2017). The enormous adaptability of *P. aeruginosa* greatly facilitates its capacity to cause chronic infections. The variability and flexibility of the pathogenicity of *P. aeruginosa* is shaped by the fact that it has a large number of virulence factors and rapidly adapts to stress factors (Jurado-Martín et al., 2021). The pathogenicity of *P. aeruginosa* consists of virulence factors that act both intracellularly and extracellularly (Balasubramanian et al., 2013). These virulence factors cause *P. aeruginosa* to proliferate, survive, and cause disease, especially without being affected by the immune responses formed in the host. It causes inflammation of the patient's lung tissues and serious tissue damage, especially during lung infections (Sadikot et al., 2005). The virulence factors of *P. aeruginosa* are, respectively, lipopolysaccharides, alginate, flagella, pili, exotoxin A, pyoverdin, pyocyanin, phospholipase C, Quorum sensing, and rhamnolipid (Jurado-Martín et al., 2021).

Lipopolysaccharide

Lipopolysaccharide (LPS) in the bacterial membrane is an important virulence factor in Gram-negative pathogenic bacteria. This LPS structure in the cell membrane consists of three different structures: lipid A, core region, and O-antigen or O-polysaccharide (Maldonado et al., 2016). Besides acting as a physical barrier, the LPS structure interacts with the host receptors and causes tissue damage with its endotoxic activity (King et al., 2009). Bacteria serotyping is performed with the O-polysaccharide or O-antigen structure in the LPS structure. These chains are protective against complement lysis and also show resistance to antimicrobial proteins. Lipid A structure in this structure activates many inflammatory precursor cells and causes adhesion by binding to asialo GM1, a ganglioside receptor (Salyers et al., 1994).

Alginate

It produces alginate with a mucoid structure so that the infection caused by the pathogen *P. aeruginosa* in the host can turn into a chronic one (Cross et al., 2020). Alginate is in an exopolysaccharide structure and is also responsible for biofilm formation and stability. Alginate, also called mucoid exopolysaccharide, is the main component of the most studied *P. aeruginosa* biofilms (Ghafoor et al., 2011). Alginate production plays a role in the adhesion of the bacteria by fixing *P. aeruginosa* to epithelial cells. This situation is mostly observed in respiratory tract infections (Salyers et al., 1994). In addition, alginate weakens the host's response to bacteria and protects against phagocytosis, but also reduces the effect of antibiotics used (Wozniak et al., 2003).

Flagella (Whip)

P. aeruginosa has a polar flagella and thus, besides gaining movement, it provides chemotaxis by directing the movements of the bacteria according to the chemicals in the environment. These polar flagella, which play an important role in adhesion to the cellular surface, help the formation of the first biofilm with its ability to attach flagels (Haiko and Westerlund-Wikström, 2013; Fooladi et al., 2013). The flagella of *P. aeruginosa* are approximately 25 nanometers (nm) in diameter and consist of 20 different parts in total. This structure consists of a filament made of polarized flagellin, a cap protein, hook and hook attachment proteins, and a series of basal bodies. Flagella are connected to the cell membrane by basal trunk pathways and take a long spiral shape (Karaderi and Kahraman, 2017). Although *P. aeruginosa* swarms on solid surfaces, flagella are primarily responsible for swimming through corkscrew rotation in aqueous or low-viscosity environments, and generate a force that moves the bacteria forward (Sampedro et al., 2015).

Pili (Fimbriae)

P. aeruginosa has hair-like appendages that enable it to adhere to surfaces and move. These extensions are called pili (fimbriae) and are short surface structures. Pili helps *P. aeruginosa* spread rapidly by colonizing the respiratory tract (Kipnis et al., 2006; Jacobsen et al., 2020). Bacteria have 4 different pili, namely Type I, Type II, Type III and Type IV. Type IV pili in bacteria are 5-8 nm in diameter and are responsible for adherence, biofilm formation, motility and adhesion. *P. aeruginosa* uses Type IV piles to provide motility, adhesion, and colonization and thus initiates the infection (Leighton et al., 2015; Burrows, 2012).

Protein Secretion Systems

Bacteria release the toxins and enzymes they produce into the outer environment with 8 different protein secretion systems (Types I, II, III, IV, V, VI, VII and IX) (Pena et al., 2019). Type I and Type V are the simplest secretory pathways and are responsible for releasing enzymes such as proteases into the external environment. (Zhao et al., 2019). Type II, Type III, Type IV, and Type VI are more complex systems and release a wide variety of exoproteins. Type III and Type IV protein secretion systems also increase the virulence of bacteria by injecting exoproteins directly into the cytoplasm of the target cell (Bleves et al., 2010; Sana et al., 2016; Pena et al., 2019). The most important secretion system is the Type III secretion system, which is used to disable and destroy the host's immune system (Anantharajah et al., 2016). Thanks to the type III protein secretion system, the formation of a bridge between two cells is provided and effector proteins are transmitted to the cytoplasm of the eukaryotic cell. Type III protein secretion system also secretes S, T, Y and U exoenzymes (Kipnis et al., 2006). *P. aeruginosa* uses all protein secretion systems effectively and thus attacks the host and causes chronic infections various toxins and hydrolytic enzymes (Pena et al., 2019). Table 2 shows that exoenzymes secreted by the Type III protein secretion system in *P. aeruginosa* and their functions.

Exotoksin A

Exotoxin A is an adenosine diphosphate-ribosyl transferase, which is secreted into the extracellular space through the Type II protein secretion system and has a toxic effect on body tissues (Lederberg, 2000). Exotoxin A is an important virulence factor for *P. aeruginosa* (Javanmardi et al., 2019). The toxin of interest is subdivided into three structurally prominent domains and one minor subdomain. The N-terminal domain, which is composed of antiparallel threads, is responsible for attachment to host cells. The middle domain, which consists of six α-helices, has membrane translocation activity. The third part, called the C-terminal domain, is the toxic part. There is a small Ib subdomain located between the mid-domain and the C-terminal domain and does not affect on toxin activity (Michalska and Wolf, 2015).

Exotoxin A causes death in experimental animals even in very small doses. Bacteria release exotoxin A into the extracellular space via the type II secretion system. Exotoxin A shows its effect by inhibiting protein synthesis in cells. Exotoxin A secreted during infection plays an important role in the formation of tissue damage by suppressing the host response. In addition, it is known that exotoxin A has an immunosuppressive effect on lymphocytes (Lederberg, 2000).

Pyoverdine

Pyoverdine is a virulence agent that ensures the transport of iron in bacteria and ensures proliferation. In addition to binding the iron required for the metabolism of *P. aeruginosa*, important virulence factors such as Exotoxin A and endoprotease are involved in the regulation of secretion (Song et al., 2010).

Table 2. Type III secretion system toxins and their functions

Secretion system	Functions
Exoenzyme S	This toxin causes cellular apoptosis. It causes tissue damage, especially in lung infections, leading to the spread of bacteria (Nicas ve Iglewski, 1985).
Exoenzyme T	It is a toxin that inhibits the uptake of *P. aeruginosa* by macrophages (Shaver and Hauser, 2004).
Exoenzyme Y	It is the second most common exotoxin of *P. aeruginosa* and inhibits the production of proinflammatory cytokines from the macrophage and epithelial cells of the host (Javanmardi et al., 2019; He et al., 2017).
Exoenzyme U	This toxin, which can be found in *P. aeruginosa*, is among the potent cytotoxins such as phospholipase and destroys various cells (Mitov et al., 2010).

Pyocyanin

Pyocyanin, belonging to the class of tricyclic phenazine compounds, is a zwitterion that contains a phenol group and exhibits weak acidic properties. In recent years, it has attracted attention as an important virulence factor produced by *P. aeruginosa*. Its low molecular weight and zwitterionic properties enable the toxin to easily cross the cell membrane (Hall et al., 2016; Fothergill et al., 2007; Lau et al., 2004; Reszka et al., 2004).

The virulence factors enhancing the effect of pyocyan on *P. aeruginosa* is stated below.

- It allows the toxin to easily penetrate the cell membrane (Hall et al., 2016; Zeng et al., 2020).
- It triggers oxidative stress by increasing the amount of intracellular perocytes (Hall et al., 2016; Zeng et al., 2020).
- It causes cell lysis by damaging various enzymes and DNA of the target cell (Hall et al., 2016; Zeng et al., 2020).
- Pyocyanin causes apoptosis of neutrophils by increasing mitochondrial Reactive Oxygen Species (ROS) formation in neutrophils (Managò et al., 2015).
- It contributes to lung colonization of *P. aeruginosa* by disrupting of epithelial tissue and increased mucous secretion in the respiratory tract (Hall et al., 2016).
- It alters the host immune response by increasing the production of interleukin-8 (IL-8) from macrophages and neutrophils (Hall et al., 2016).
- In addition to inhibiting catalase activity, it also reduces transcription of genes encoding catalase (O'Malley et al., 2003).
- It is a redox active secondary metabolite responsible for the blue-greenish color of *P. aeruginosa* colonies in culture (Gellatly and Hancock, 2013).

Rhamnolipid

Discovered in 1949, rhamnolipids are amphipathic extracellular secondary metabolites (Jarvis and Johnson, 1949; Alfiniyah et al., 2019). In addition, thanks to the rhamnolipid secreted by *P. aeruginosa*, the surfactant structure in the lung tissue deteriorates. Tight junctions in the respiratory epithelium are disrupted as a result of the subsequent decrease in transepithelial electrical resistance. It contributes to the formation of the biofilm layer by increasing the release of LPSs to the cell surface at low or normal concentrations of rhamnolipids. However, as a result of the overproduction of rhamnolipids, biofilm formation is prevented (Zulianello et al., 2006; Nickzad and Déziel, 2014; Köhler et al., 2010).

Phospholipase C

Phospholipase C is an enzyme that hydrolyzes the ester bond between phosphoric acid and glycerol in glycerophospholipids and is a heat-stable thermolabile hemolysin (Khalifa et al., 2011). Phospholipase C is secreted from the Type 2 secretion system and breaks down phospholipids in the membrane of the target cell and sphingomyelin, which has hemolytic activity (Kipnis et al., 2006).

Quorum Sensing

Quorum sensing system is known as a communication mechanism. Bacteria determine their density by measuring the signals generated by the related system and other bacteria (Venturi, 2006; Chu et al., 2015). Such communication between cells plays an important role in the formation of biofilms and the initiation of infection (De Kievit, 2009; Davies, 2003). The induction of stimulating signal proteins bound to the membrane of bacterial cells allows the establishment of bridges between bacterial cells and then the formation of bacterial colonies on the surface (Hall-Stoodley et al., 2004; Şahin and Kaleli, 2018).

The Epidemiology of *Pseudomonas Aeruginosa*

P. aeruginosa is a pathogenic microorganism found on the surface of foods such as fruits and vegetables. It settles in the gastrointestinal tract after food consumption. *P. aeruginosa*, which can colonize in the small intestines, can also cause temporary colonization in the large intestine. This microorganism has a high ability to adapt to different environmental conditions. The reason for this is that it can multiply in different environments with minimum nutritional requirements (Brady, 2009).

Epidemiological studies are concerned with the possibilities, sources and mechanisms of transmission of *P. aeruginosa* to patients in the hospital setting. Environments where bacteria are found include solutions, creams, faucets, sink drains, incubators, personnel, and inhalation and resuscitation equipment (Lowbury et al., 1970). *P. aeruginosa* is reported at a rate of 13.2–22.6% in intensive care units and is responsible for 11-13.8% of all nosocomial infections (Weinstein et al., 2005; Kim et al., 2000).

P. aeruginosa can be found on the skin, nasal mucosa, throat and normal flora of healthy people (Shannon and French, 2004). There are three stages in the development of the infection. These; colonization, invasion and systemic spread (Bergagne, 2004). During infection, the degree of infection depends on the host's defense mechanism and bacterial virulence factors. In this way, it is determined whether there will be systemic spread at the colonization stage or not. *P. aeruginosa* is an important pathogen in patients with both primary and acquired immunodeficiencies (Lee et al., 2006). In addition, it is an important cause of bacteremia in patients with acute leukemia and is responsible for 14-21% of bacteremia attacks in these patients (Chatzinikolaou et al., 2000). *P. aeruginosa* plays an important role in patients with Cystic Fibrosis, where chronic and recurrent infections of the respiratory tract are common (Burns et al., 2001).

Antibiotic Resistance and Formation of Biofilm in Pseudomonas aeruginosa

Antibiotic Resistance

Antibiotic resistance is the ability of an organism to resist the action of an antimicrobial agent to which it was previously sensitive (Bagge et al., 2004). Thanks to the existing resistance to many antibiotics and antiseptics, *P. aeruginosa* is among the most common pathogens seen in hospitals and intensive care units (Santajit and Indrawattana 2016).

One of the most notable distinctions of *P. aeruginosa* from other bacteria is its exceptionally low cell membrane permeability. For example, *Escherichia coli* shows intrinsic resistance to antimicrobial agents such as β-lactam and penem group antibiotics because it is 1/100 times more

selective than the outer membrane permeability (Martin-Loeches et al., 2013). In addition to the intrinsic resistance mechanism, it also creates resistance through some mechanisms such as a flow system that expels the antibiotic from the bacterial cell and the production of antibiotic inactivating enzymes (Poole, 2005). In many studies, it has been observed that *P. aeruginosa* strains develop antibiotic resistance during antibacterial treatment and thus remain viable (Rello et al., 2006; Dietz et al., 1997; Pachori et al., 2019).

Biofilm Formation

Biofilm formation is an integral part of the microbial life cycle in nature. In the food processing process, the transmission route of bacteria primarily occurs through non-compliance with hygiene rules and cross-contamination of raw or undercooked foods. Foodborne pathogens form biofilms as a survival strategy in a variety of adverse environments. In this way, they cause recurrent contamination and food-borne infections and intoxications in the food business (Bai et al., 2021). A biofilm is a community of microbes that typically live on surfaces and are contained within an extracellular matrix. *P. aeruginosa* is a microorganism famous for developing robust biofilms that are highly resistant to antibiotics, disinfectants, and host defenses, disrupting bacterial clearance (Lee and Yoon, 2017). *P. aeruginosa* is an opportunistic microorganism that can attach to food or food contact surfaces and form a biofilm. It is difficult to eradicate because the growth of the agent occurs in a biofilm and it is resistant to antibiotics and disinfectants (Sırıken and Öz, 2017). Studies have reported that biofilm formation occurs in five stages. These stages include:

- In the first stage of biofilm formation, bacteria control whether oxygen, osmolality, nutrient concentrations and temperature factors are at appropriate levels among environmental factors. They are then reversibly attached to the surface using extensions such as pili and flagella (Garrett et al., 2008).
- After the first contact on the surface, in the second stage, bacteria form stronger irreversible bonds thanks to components such as exopolysaccharide matrix (Branda et al., 2005).
- After microorganisms become stable by attaching to a biotic or abiotic surface, they initiate a proliferation and division process initiated by certain chemical signals in extracellular polymeric Extracellular Polymeric Substance (EPS) materials. This process then leads to the formation of micro-colonies (Costerton et al., 1999).
- At this stage, cells communicate with each other with the help of auto-inducing signals, increase the microbial cell density and begin to form three-dimensional structures (Parsek and Singh, 2003).
- At this stage, the microbial cells in the biofilm undergo rapid proliferation and spread to transform from the dormant sessile form to the motile form. Thus, the separated microbial cells allow the biofilm layer to spread to the environment (Donlan and Costerton, 2002; Jamal et al., 2018; Olivares et al., 2020).

The biofilm layer formed when *P. aeruginosa* becomes infected is somewhat difficult to treat. Because the antibiotic-tolerant cells in the biofilm layer are not affected by the treatment or can re-proliferate in the biofilm by reducing its effect (Akiyama et al., 2017). In the food sector, polymicrobial biofilms are formed on the surfaces by *P. aeruginosa*. Thus, it helps the persistence of many foodborne pathogens and raises concerns in terms of food safety and public health (Bai et al., 2021).

Isolation and Identification of Pseudomonas aeruginosa

Various methods have been developed to isolate *P. aeruginosa* (Lambe and Stewart, 1972). The most important media used in classical cultivation methods are *Pseudomonas* CN (Cetrimide) Agar and *Pseudomonas* CFC (Cephaloridine, Fucidin, and Cetrimide) Agar. These media are left to incubate for 24-48 hours at a temperature of 30-37 °C, where *P. aeruginosa* ideally grows. After the incubation, the pyocyanins formed by the suspicious colonies are examined in ultraviolet light, and the reddish brown colonies are treated as suspicious and biochemical tests are performed. Although blue-green colonies are accepted as *P. aeruginosa*, they should be subjected to biochemical tests (Kristiansen, 1983).

Biochemical analyses used in the isolation and identification of *P. aeruginosa* are performed on 5% blood agar, beta hemolysis, catalase, oxidase and motility tests, methyl red, Voger-Proskauer, Indole, H_2S, xylose, gram staining and citrate tests (Kleeberger and Busse, 1975; Paul and Sinha, 2017). The biochemical test results of *P. aeruginosa* are shown in Table 3. (Ezemba et al., 2022).

Table 3. Identification of P. aeruginosa isolates (Ezemba et al., 2022).

Biochemical Tests	*Pseudomonas aeruginosa*
Catalase test	+
Oxidase test	+
Motility test	+
Citrate test	+
Methyl Red test	-
Gram staining	-
Xylose test	-
Indole test	-
Hydrogen Sulfide (H_2S) test	-
Voges-Proskauer	-

In addition to microbiological cultivation methods, *P. aeruginosa* in foods is also confirmed by molecular methods. Polymerase Chain Reaction (PCR) based molecular techniques are used to detect specific genes of P. aeruginosa. *16S rDNA, 16S-23S rDNA ITS, ETA, fliC, algD, oprI, oprL, toxA, gyrB and ecfX, lasB, phzM, toxA, ExoU* and *ExoS* gene regions are generally searched for the detection and identification of *P. aeruginosa*. The genomic DNA of *P. aeruginosa* is extracted from bacterial colonies by the set buffer method. After performing the necessary PCR procedures, the 16S rRNA gene sequence of *P. aeruginosa* is compared with those in the NCBI/Eztaxon Ribosomal Database Project (RPD) and EMBL nucleotide sequence databases using the BLAST (blastn) program (Wei et al., 2020). There are also molecular typing methods to identify P. aeruginosa transmission sources and routes. These; Pulsed-field gel electrophoresis (PFGE) and

variable-number tandem repeat (VNTR) are current molecular epidemiological typing systems such as amplified fragment length polymorphism analyses (Eckmanns et al., 2008; Turton et al., 2010). Using both Roche 454 and Illumina, which are new generation sequencing systems, the whole genome sequence of the *P. aeruginosa* isolate can be determined (Snyder et al., 2013).

Presence of *Pseudomonas aeruginosa* in Foods

P. aeruginosa can be found as a spoilage factor in various foods such as meat, milk, vegetables and fruits, especially in water (Gram et al., 2002). *P. aeruginosa* causes major problems for the food industry (Collins et al., 1989).

Detection of P. aeruginosa in meats

P. aeruginosa is the most common psychrotrophic organism that causes spoilage in aerobically stored foods with high water content and natural pH, especially beef and poultry (Gram et al., 2002). *P. aeruginosa* limits the shelf life of chicken meat in cold storage by creating slipperiness and unpleasant odor on the surface (Lopez et al., 2015). *P. aeruginosa*, which multiplies easily at refrigerator temperature, causes significant economic losses by causing deterioration of beef in cold storage (Liao et al., 2019).

In a study on beef and poultry, 86 *Pseudomonas* spp. isolates were obtained. As a result of the biochemical tests performed for the identification of these strains isolated from beef, 3 (3.49%) of them were found to be *P. aeruginosa*. *Pseudomonas* spp. obtained from chicken meat. It was stated that none of the isolates were *P. aeruginosa* (Akan and Gürbüz, 2016).

In another study, 110 *Pseudomonas* spp. isolated from pork and beef spoiled under aerobic conditions. 13 of the species were identified as *P. aeruginosa* (Shaw and Latty, 1982).

Elbehiry et al. (2022) stated that only 3 of the 69 *Pseudomonas* species they detected in a total of 320 chicken meats were *P. aeruginosa*.

In a study conducted in the Alborz province of Iran, 29 (7.83%) of 370 samples from raw, frozen and imported beef were found to be contaminated with *P. aeruginosa* (Rezaloo et al., 2022).

In the study of Poursina et al. (2022), 350 meat samples were taken, 175 of which were beef and 175 were sheep. *P. aeruginosa* was detected in 13 of the beef samples (7.42%) and in 10 of the sheep meat samples (5.71%). In total, *P. aeruginosa* was found in 23 of 350 meat samples (6.57%).

Detection of P. aeruginosa in milk and dairy products

P. aeruginosa contamination causes significant problems in the dairy industry (Dhanashekar et al., 2012). *P. aeruginosa* is among the most frequently isolated bacteria from surfaces in the food industry in general. Biofilm layers caused by *P. aeruginosa* may form on the inner surface of milk cooling tanks and pipelines before heat treatment in the enterprise (Marchand et al., 2012). When *P. aeruginosa* is mixed with milk, it multiplies very quickly and causes changes in the color, smell, structure and consistency of milk (Şen and Halkman, 2006). Enzymes such as esterase secreted by *P. aeruginosa* in cold stored milk become active again after pasteurization because they are heat resistant. Therefore, they can cause bitterness by causing the breakdown of triglycerides in products such as milk, cheese, cream and butter (Cousins and Bramley, 1983).

In a study, 45 *Pseudomonas* spp. isolates were obtained from raw milk. It was determined that only 4 (8.88%) of the isolates were *P. aeruginosa* (Jooste et al., 1986).

In another study, 11 (22.9%) of the *Pseudomonas* isolates obtained from 48 milk samples were found to be *P. aeruginosa* (Uraz and Çıtak, 1998).

Arslan et al. (2011) reported that 32 *Pseudomonas* spp. isolates were obtained. of these isolates, 15% were *P. pseudoalcaligenes*, 5% *P. alcaligenes*, 0.7% *P. fluorescens* biovar V, 0.7% *P. pseudoalcaligenes* subspecies citrulli and 1.4%. was also identified as *P. aeruginosa*.

Okuno et al. (2021) reported that conducted in Rio de Janeiro, Brazil, the presence of *P. aeruginosa* was investigated in 33 traditional Minas cheeses. As a result, *P. aeruginosa* was detected in 4 (12.1%) of the samples.

Detection of P. aeruginosa in water sources

P. aeruginosa is an pathogen that grows in marine habitats and marshes (Garvey et al., 2018).

In a study, 7904 water samples obtained from various water sources were examined for the presence of *P. aeruginosa*. As a result, *P. aeruginosa* was detected in 524 (6.6%) of the examined water samples. Of the samples, 243 are from hot pool water, 51 of them (21%), 40 of them are tap water, 3 of them (8%), 5811 of them are from jacuzzis, 432 of them (7.4%) are from swimming pools, 270 of them are from swimming pools and 5 of them (2%) are from swimming pools, 67 of them are bottled natural mineral waters, 2 of them (3%) and 1234 of them are port irrigation hydrates, and 24 of them (2%) were found to be contaminated with *P. aeruginosa* (Caskey et al., 2018).

It was observed that 19 (7.6%) of 251 water samples obtained from fountains in common areas in Brazil were contaminated with *P. aeruginosa* (Anversa et al., 2019).

As a result of a study conducted with samples taken from wastewater treatment plants, 40 *Pseudomonas* spp. isolates were obtained. It was determined that 22 (55%) of the 40 isolates identified were *P. aeruginosa* (Keloğlu et al., 2019).

Xu et al. (2019) reported that conducted in different water samples, *P. aeruginosa* was detected in 3% of drinking water, 9% of tap water, 18.8% of bottled water and 90% of sewage water.

In a study conducted in India, the microbiological quality of the waters in the water tanks in 32 different villages was investigated. According to the results obtained, it was determined that 17 samples (53.1%) contained *P. aeruginosa* (Rizvi and Mohammed-Aslam, 2019).

In a study conducted in China, 314 drinking water and 133 mineral water samples were collected from 23 cities. *P. aeruginosa* was found positive in 77 (24.5%) of the obtained drinking water samples and in 18 (13.5%) of the mineral water samples (Wei et al., 2020).

Detection of P. aeruginosa in vegetables

P. aeruginosa is a ubiquitous bacterium. It is commonly found in foods. In vegetables (Hardalo and Edberg, 1997), *P. aeruginosa* is frequently seen especially in tomato, eggplant, spinach, celery, onion, carrot, lettuce and cucumber (Shooter et al., 1971; Xu et al., 2019).

In a study using molecular methods, 26 untreated vegetable samples consisting of chard, lettuce, green beans, potatoes, zucchini, cucumbers and onions were examined for P. aeruginosa. As a result, *P. aeruginosa* was found in 18% of the samples (Ruiz-Roldán et al., 2021).

In a study in which 38 raw vegetable samples consumed in hospital meals consumed by inpatients in the oncology department of a University Hospital were examined for *P. aeruginosa*, it was found in 19% of the vegetable samples. Among the vegetable samples, lettuce, chicory and watercress were the most active (Correa et al., 1991).

Conclusion

P. aeruginosa, is a very flexible and changeable microorganism that adapts to various living conditions. This microorganism becomes important in food businesses as it can easily survive in foods such as meat and meat products, milk and dairy products, water, fruits and vegetables. Besides being an opportunistic pathogen, it is among the best-known biofilm-forming bacteria. It is very easy to stick as a result of contact with the surface of the food. Therefore, it is a microorganism that is included in the scope of food safety among *Pseudomonas* species. *P. aeruginosa* causes difficult-to-treat infections with high incidence and various virulence factors. In recent years, the effectiveness of antimicrobials in the treatment of *P. aeruginosa* infections has gradually decreased. Infections are difficult to eradicate due to high levels of antibiotic resistance and their growth in biofilms. Therefore, unnecessary use of antibiotics in this bacterium should be abandoned and different treatment methods should be developed. Personnel working in food establishments should be made aware, and surfaces (floor, wall, counter, etc.) and cracks that may form biofilm should be cleaned and renewed well by paying attention to Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) practices.

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A Review of the Nutritional Profile, Chemical Composition and Potential Health Benefits of *Aronia melanocarpa* (Chokeberry) Berries and Products

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ABSTRACT

Aronia melnocarpa, commonly known as chokeberry, originates from the eastern region of North America and belongs to the *Rosaceae* family within the *Maloideae* subfamily. The sour taste of fresh chokeberries makes them infrequently eaten as is, but they find extensive use in the food industry for creating fruit juices, fruit teas, wines, jams, jellies, and dietary supplements. Chokeberries represent a source of a wide range of bioactive compounds with potential health benefits for humans. Among the effects supporting human health are antidiabetic, anti-inflammatory, antimicrobial, and anticancer properties, as well as protection for the heart, liver, and nervous system. The abundant presence of polyphenols, such as phenolic acids, flavonols, anthocyanins, flavanols, and proanthocyanidins, plays a crucial role in conferring the remarkable bioactivity of chokeberries. These compounds are responsible for many of the health benefits associated with the consumption of chokeberries. Chokeberry fruits and their derived products showcase notable antioxidant properties and have the potential to promote health by effectively reducing the formation of free radicals. In this review, a comprehensive analysis of scientific research has been conducted to explore the polyphenolic compounds found in chokeberries, as well as their antioxidant potential. The findings in this review are likely to have a significant impact on future research focused on developing functional food products based on chokeberries. Chokeberries possess the potential to serve as food constituents intentionally crafted to augment antioxidant capacity. However, similar to other natural plants and medicinal products, conducting extensive research is crucial to assess the antioxidant potential, safety, and mechanisms of action of chokeberries. Therefore, the aim is to make a positive contribution to the continuation of research on the positive effects of chokeberry on health.

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Introduction

Aronia (chokeberry) is a member of the Maloideae subfamily within the Rosaceae family. Its origin can be traced back to the eastern regions of North America, and it comprises two species: *Aronia melanocarpa* (Michx.) Elliot (black) and *A. arbutifolia* (red). *A. prunifolia* (purple) is a third species that is a hybrid of the other two (Kulling and Rawel, 2008). Out of these three options, black chokeberry stands out as the most valuable and beneficial as a food source (Ćujić et al., 2018). This species prefers sunny and well-lit areas but can tolerate partial shade and short periods of drought. Black chokeberries have a limited tolerance to excessive soil moisture, which necessitates the implementation of drip irrigation during hot and dry periods (Negreanu-Pirjol et al., 2023).

Because of its cold-hardiness, *Aronia* can be grown not only in temperate climates but also in regions with temperatures as low as below -35°C (Ćujić et al., 2018). *Aronia* plants do not have specific requirements concerning climatic and ecopedological factors, and they are not prone to severe disease or pest attacks (Negreanu-Pirjol et al., 2023). Their adaptability to conditions unsuitable for other fruits makes them a common choice for ornamental purposes in gardens (Ćujić et al., 2018). *Aronia* was highly valued by Native Americans and was traditionally used to make tea for treating colds (Kulling and Rawel, 2008). Currently, *Aronia* is predominantly cultivated and utilized for the production of various products, including fruit juices, jams, liqueurs, wines, and food colorants. Consistent intake of antioxidant-rich vegetables, fruits and other food items is widely recognized to enhance overall health and is linked to a reduced risk of various chronic diseases. The beneficial health effects of plant-based foods primarily stem from the presence of bioactive compounds within them. Phytochemicals,

commonly found in these plant-based foods, can exert protective effects against the onset of diverse diseases, either individually or through collaborative interactions known as synergistic effects (Ćujić et al., 2018).

The fruit and leaves of *A. melanocarpa* contain a significant abundance of diverse bioactive compounds, with a primary focus on polyphenolic compounds, along with vitamins and minerals. As a consequence, *Aronia* demonstrates a broad spectrum of beneficial health effects (Szopa et al., 2017; Jurikova et al., 2017).

Around 25% of the polyphenolic content in *Aronia* fruits consists of anthocyanins, with cyanidin derivatives being the most prevalent among them. The principal flavonol found in *A. melanocarpa* is quercetin. Essential constituents of *A. melanocarpa* encompass chlorogenic and neochlorogenic acids, alongside tannins. All these factors contribute to *Aronia* surpassing known antioxidant sources such as grapes, cranberries, blueberries, elderberries, or currants (Negreanu-Pirjol et al., 2023). The polyphenolic compounds in *A. melanocarpa* exhibit not only high antioxidant capacities but also antimicrobial, anti-inflammatory, antiviral, anticancer, and antidiabetic properties (Borowska et al., 2016; Kokotkiewicz et al., 2010; Kulling and Rawel, 2008; Jurikova et al., 2017; Szopa et al., 2017).

Aronia stands out among plant-based foods due to its high polyphenol content and potential health effects. The purpose of this study is to review the chemical composition and nutritional profile of *A. melanocarpa* fruits, fruit juice and pomace, providing essential information for the development of beneficial components and emphasizing their advantageous health properties.

Aronia's Nutritional Composition and Health-Beneficial Components

The chemical makeup of chokeberry fruit is influenced by several factors, including soil composition, climate conditions, grain maturity, storage conditions and harvesting techniques. It contains numerous compounds, including organic acids, carbohydrates, amino acids, minerals, polyphenols, vitamins and aroma compounds (Tolić et al., 2017; Kulling and Rawel, 2008).

Dark-colored fruits are widely acknowledged as significant providers of anthocyanins. Chokeberry, like other dark-colored fruits, is an essential reservoir of anthocyanins. *Aronia* is rich in health-beneficial compounds classified as antioxidants, including proanthocyanidins, flavonols, anthocyanins, flavanols and phenolic acids, which contribute to its status as a biologically active and polyphenol-rich source (Pliszka et al., 2008; Du et al., 2004; Ding et al., 2006; Zhang et al., 2021). Besides their antioxidant activity, polyphenols also play roles as carriers of characteristic taste, aroma, color, and nutritional value (Robards et al., 1999).

Table 1. Chemical composition of Aronia berries, juice and pomace.

	Berries	Juice	Pomace
pH	3.36–3.79 [Tanaka et al. 2001], 3.3–3.7 [Lancrajan et al. 2012], 3.23–3.57 [Skrede et al. 2012]	3.77–3.96 [Tolić et al. 2017], 3.54–3.92 [Tolić et al. 2015], 3.5 [Handeland et al. 2014], 3.42–3.72 [Sosnowska et al. 2016], 3.15–3.45 [Bolling et al. 2015], 3.5 [Daskalova et al. 2015]	
Titratable acidity	0.75–1.05 g/100 g [Ochmian et al. 2012], 1.42 g/100 g [Ochmian et al. 2009], 0.493–0.548 g/100 g [Skupień et al. 2007], 6.7–11.9 g/kg [Šnebergrová et al. 2014], 1.9–2.6 g/kg [Wichrowska et al. 2007], 1.03–1.44 g/100 g [Skrede et al. 2012], 1.24–1.31 g/100 g [Skupień et al. 2008]	0.89–1.06% [Tolić et al. 2017], 0.29–1.32% [Tolić et al. 2015], 0.85–1.22% [Bolling et al. 2015], 0.89% [Daskalova et al. 2015]	0.52–0.58% [Sójka et al. 2013]
Dry matter	15.30–19.50% [Ochmian et al. 2012], 17.9–26% [Mayer-Miebach et al. 2012], 26.67–30.76% [Skupień et al. 2007], 15.7% [Lancrajan et al. 2012], 22.14–23.45% [Wichrowska et al. 2007], 21.0–26.0% [Andrzejewska et al. 2015], 18.3–23.5 g/100 g [Skrede et al. 2012], 18.92–20.14% [Skupień et al. 2008], 15–31 [Skupień et al. 2007; Ochmian et al. 2012]	11–17 [Mayer-Miebach et al. 2012], 19.22–26.94% [Tolić et al. 2017], 11.1–17.4% [Mayer-Miebach et al. 2012], 15.46–16.87% [Oszmiański et al. 2016], 13.42–21.54% [Tolić et al. 2015], 18.1% [Daskalova et al. 2015]	45–50% [Mayer-Miebach et al. 2012], 90.21% [Pieszka et al. 2015], 93.6–94.9% [Sójka et al. 2013], 90.8% [Nawirska et al. 2004]
Dietary fiber	56 g/kg [Kulling et al. 2008]	0.3 g/100g [Yamane et al. 2017; Yamane et al. 2016]	21.79% [Pieszka et al. 2015], 63.5–77.9% [Sójka et al. 2013], 72.0% [Wawer et al. 2006]
Fat	0.09–0.17 g/100g [Tanaka et al. 2001] 0.14 g/100 g [Kulling et al. 2008]	<1 g/L [Yamane et al. 2017]	5.15% [Pieszka et al. 2015], 2.9–13.9% [Boncheva et al. 2013]
Protein	3.71 g/100 g [Červenka 2011], 0.60–0.81 g/100g [Tanaka et al. 2001], 0.7 g/100g [Lancrajan 2012]	2 g/L [Yamane et al. 2017]	%4.9–24.1 [Sójka et al. 2013], 10.77% [Pieszka et al. 2015]
Total Sugar	9.16–13.79 g/100 g [Ochmian et al. 2012], 68–158 g/kg [Mayer-Miebach et al. 2012], 19.32–20.92 g/100 g [Skupień et al. 2007], 83.0–111.6 g/kg [Wichrowska et al. 2007], 6.21–6.91 g/100 g [Skupień et al. 2008]	89.49–162.37 g/L [Sosnowska et al. 2016], 12.0–19.6 g/100 mL [Handeland et al. 2014], 110–143 g/L [Mayer-Miebach et al. 2012]	84 g/kg [Mayer-Miebach et al. 2012]

Dietary fiber

Dietary fibers are essential compounds found in foods. They have the potential to bind harmful substances and heavy metals in the body, thereby reducing their levels (Nawirska et al., 2005). The consumption of high glycemic index foods is thought to play a substantial role in the emergence of various metabolic disorders. (Hauner et al., 2012). High intake of dietary fiber (DF) can help mitigate these risks (Stephen et al., 2017; Lovegrove et al., 2017).

A. melanocarpa fruits contain 56 g/kg or 5.6 g/100 g FW (fresh weight) of dietary fiber (Kulling and Rawel, 2008), while the fiber content in *Aronia* pomace varies between 21.79% and 77.9% DM (dry matter) (Pieszka et al. 2015; Sójka et al. 2013; Wawer et al. 2006). Data presented by Sójka et al. (2013) indicate that chokeberry pomace is characterized by significant cellulose (35%), hemicellulose (34%), lignin (24%), and pectin (8%) content. Rich in dietary fiber, *A. melanocarpa* by-products are considered valuable sources for food supplements and functional foods (Wawer et al., 2006).

Fat

According to research, *Aronia* fruits have a total fat content of 0.09–0.17 g/100g (Tanaka et al., 2001; Kulling and Rawel, 2008). The fat content in *Aronia* pomace ranges from 2.9% to 13.9% DM (Pieszka et al., 2015; Sójka et al., 2013). Studies have shown that the fat obtained from *A. melanocarpa* and its seeds is rich in tocopherols, sterols, and phospholipids. The dried pulp and seeds exhibit a fatty acid combination with a substantial presence of polyunsaturated fatty acids, with linoleic acid being the primary fatty acid, constituting approximately 73.6% of the total fatty acids (Zlatanov et al., 1999; Pieszka et al., 2015).

Protein

The protein content in *Aronia* fruits is generally low and falls within the range of 0.60–3.71 g/100g (Červenka et al., 2011; Tanaka et al., 2001; Lancrajan et al., 2012). Regarding pulp and dried pulp, the reported total protein content ranged from 4.9% to 24.1% and was found to be 10.77%, respectively. The most abundant amino acids in dried pulp were aspartic acid, arginine, and glutamic acid (Pieszka et al., 2015). In addition, research has shown that the fraction without seeds of the pulp has a considerably lower protein content when compared to the seed fraction (Sójka et al., 2013; Pieszka et al., 2015).

Sugar

Several studies conducted by different researchers have reported that the total sugar content in *Aronia* fruits falls within a range of 6.21 to 20.92 g per 100 grams (Ochmian et al., 2012; Mayer-Miebach et al., 2012; Skupień et al., 2007; Wichrowska et al., 2007; Skupień et al., 2008). In *Aronia* juice, the total sugar content has been found to be between 8.94 and 19.6 g/100 mL (Sosnowska et al., 2016; Handeland et al., 2014; Mayer-Miebach et al., 2012). For *Aronia* pomace, a sugar content of 84 g/kg was reported (Mayer-Miebach et al., 2012).

Minerals

Minerals and trace elements hold a crucial role in cell metabolism and are instrumental in activating enzymes that participate in antioxidant systems (Skupień et al., 2008). High intake of potassium (K), calcium (Ca), magnesium (Mg) and has been associated with reduced risk of osteoporosis, stroke, and hypertension (Oszmiański and Lachowicz, 2016). Iron and other trace minerals are fundamental constituents of various compounds responsible for oxygen transport and storage systems in the body. Moreover, they act as cofactors for enzymes, facilitating their proper functioning (Oszmiański and Lachowicz, 2016; Tolić et al., 2015; Daskalova et al., 2015). Molybdenum (Mo), as a micro-nutrient, serves as a structural component of xanthine dehydrogenase and xanthine oxidase enzymes. These enzymes have a significant function in the urea synthesis pathway (Jakobek et al., 2012a).

Based on previous research, Table 2 presents the micro and macro element contents in fresh chokeberry fruit, pomace and juice. The dominant macro elements reported by the authors are potassium and calcium, while phosphorus and magnesium are other elements showing relatively high concentrations. Macro elements play a crucial role in the control and regulation of metabolism. Also, micro-elements have essential biological functions as integral constituents of enzymes or protein structures. They are involved in various essential functions, including oxygen storage, electron transport, metal transport, redox processes, and a wide range of biochemical reactions (Romani et al., 2016). Regarding micro elements in *Aronia* and its products, the highest values were found for iron (Fe) and manganese (Mn).

Polyphenols

Blackberries, whole grains, a variety of vegetables, grapes and numerous other fruits are abundant sources of polyphenols, being classified chemically as polyphenolic compounds. (Zhang et al., 2021). Polyphenols encompass various subgroups of phenolic compounds with multiple hydroxyl groups and aromatic rings (Kähkönen et al., 2001; Manach et al., 2004). Polyphenols are widely recognized as one of the most significant antioxidants present in the human diet. Chokeberry fruits stand out as a highly abundant source of polyphenols, encompassing flavanols, flavonols, anthocyanins, phenolic acids, and proanthocyanidins. (Oszmiański and Lachowicz, 2016; Denev et al., 2013; Dudonné et al., 2015; Gramza-Michałowska et al., 2017). The presence of phenolic compounds in fruits is influenced not only by the plant variety but also by cultivation conditions, including factors like soil nutrient content, light exposure, temperature, water availability, and the timing of harvesting. According to researchers, the composition of polyphenols in chokeberry experiences significant modifications as the fruit development and ripens, with the greatest total polyphenol content observed in unripe fruits (Gralec et al., 2019). Polyphenols are the key bioactive components responsible for the high bioactivity exhibited by chokeberry. (Sidor and Gramza-Michałowska, 2019). It is challenging to recommend precise dosages of polyphenols to be consumed through foods (Williamson, 2017). On average, the daily intake of phenolics is estimated to be 1 g (Malik et al., 2003). The total polyphenol content determined by researchers in *Aronia* fruit, *Aronia* juice, and pomace is presented in Table 3. The polyphenol content in *Aronia* fruit was found to vary within the range of 6.03-23.40 g/kg, while in *Aronia* juice, it ranged from 2.73 to 11.24 g/L.

Table 2. Mineral composition of Aronia berries, juice and pomace

Mineral	Berries	Juice	Pomace
Ca	60–117 mg/100 g [Pavlovic et al. 2015]; 22.8–43.9 mg/100 g [Tanaka et al. 2001], 32.2 mg/100 g [Lancrajan et al. 2012], 119.0–552.3 mg/kg [Šnebergrová et al. 2014]	14–123 mg/100 g [Pavlovic et al. 2015]; 150.4 mg/L [Cindrić et al. 2017]; 138–1225 mg/kg [Pavlovic et al. 2015], 151 ppm [Handeland et al. 2014]	219–408 mg/100 g [Pavlovic et al. 2015]; 2.75 g/kg [Pieszka et al. 2015]
Mg	15.5–17.4 mg/100 g [Tanaka et al. 2001], 16.2 mg% [Lancrajan et al. 2012], 83.3–314.2 mg/kg [Šnebergrová et al. 2014], 164–578 mg/kg [Pavlovic et al. 2015]	140 mg/L [Cindrić et al. 2017]; 209–589 mg/kg [Pavlovic et al. 2015], 85 ppm [Handeland et al. 2014]	37–250 mg/100 g [Pavlovic et al. 2015]; 0.88 g/kg [Pieszka et al. 2015]
K	164–265 mg/100 g [Tanaka et al. 2001], 218 mg% [Lancrajan et al. 2012], 1356.3–3659.7 mg/kg [Šnebergrová et al. 2014], 2707–4977 mg/kg [Pavlovic et al. 2015]	5.3 mg/L [Cindrić et al. 2017]; 848–3204 mg/kg [Pavlovic et al. 2015], 1242 ppm [Handeland et al. 2014]	181–308 mg/100 g [Pavlovic et al. 2015]; 2.78 g/kg [Pieszka et al. 2015]
Na	2.0–3.7 mg/100 g [Tanaka et al. 2001], 2.6% [Lancrajan et al. 2012], 12.5–16.8 mg/kg [Pavlovic et al. 2015]	6.4 mg/L [Cindrić et al. 2017]; 19.6–56.3 mg/kg [Pavlovic et al. 2015], 19 ppm [Handeland et al. 2014]	5–9 mg/100 g [Pavlovic et al. 2015], 0.037 g/kg [Pieszka et al. 2015]
P	15.9–21.7 mg/100 g [Tanaka et al. 2001], 257.0–417.5 mg/kg [Šnebergrová et al. 2014], 239–956 mg/kg [Pavlovic et al. 2015]	167–1037 mg/kg [Pavlovic et al. 2015]	239 mg/100 g [Pavlovic et al. 2015]; 2.39 g/kg [Pieszka et al. 2015]
Se	0.21–0.28 mg/kg [Pavlovic et al. 2015]	0.07–0.1 mg/100 g [Pavlovic et al. 2015]	
Cu	0.035–0.056 mg/100 g [Tanaka et al. 2001], 0.82–2.11 mg/kg [Pavlovic et al. 2015]	0.68–4.51 mg/kg [Pavlovic et al. 2015]	0.5–1.2 mg/100 g [Sójka et al. 2013]; 1.95 mg/kg [Pieszka et al. 2015]
Fe	0.33–1.68 mg/100 g [Tanaka et al. 2001], 9.4–14.2 mg/kg [Pavlovic et al. 2015]	7.2–25.2 mg/kg [Pavlovic et al. 2015]	7.5–8.6 (mg/100 g) [Sójka et al. 2013]; 197 mg/kg [Pieszka et al. 2015]
Zn	0.090–0.220 mg/100 g [Tanaka et al. 2001], 4.09–8.40 mg/kg [Pavlovic et al. 2015]	0.89–3.45 mg/kg [Pavlovic et al. 2015]	0.6–3.7 mg/100 g [Pavlovic et al. 2015]; 15.7 mg/kg [Pieszka et al. 2015]
Mn	0.132–0.263 mg/100 g [Tanaka et al. 2001], 5.49–17.89 mg/kg [Pavlovic et al. 2015]	2.98–11.77 mg/kg [Pavlovic et al. 2015]	3.2 mg/100 g [Sójka et al. 2013]; 31.5 mg/kg [Pieszka et al. 2015]
Si	2.37–6.37 mg/kg [Pavlovic et al. 2015]	0.3–0.7 mg/100 g [Pavlovic et al. 2015]	
Cr	0.49–0.53 mg/kg [Pavlovic et al. 2015]	0.55–0.74 mg/kg [Pavlovic et al. 2015]	
Mo	0.016–0.021 mg/kg [Pavlovic et al. 2015]	0.005–0.006 mg/100 g [Pavlovic et al. 2015]	
Ni	0.143–0.740 mg/kg [Pavlovic et al. 2015]	0.130–0.860 mg/kg [Pavlovic et al. 2015]	
Pb	0.048–0.091 mg/kg [Pavlovic et al. 2015]	0.006–0.01 mg/100 g [Pavlovic et al. 2015]	
Cd	0.016–0.041 mg/kg [Pavlovic et al. 2015]	0.050–0.064 mg/kg [Pavlovic et al. 2015]	

Flavonoids and Phenolic Acids

Flavonoids are polyphenolic compounds that contain two aromatic rings linked together by a three-carbon bridge, known as C6-C3-C6 (Zhang et al., 2021). Flavonoids are divided into various subclasses, each highlighting important chemical properties. *A. melanocarpa* fruits are abundant in compounds like proanthocyanidins, phenolic acids, and anthocyanidins. However, it is noteworthy that they have a notably low level of flavonols. (Negreanu-Pirjol et al., 2023). Numerous studies have demonstrated that chokeberries possess a relatively low flavonol content, comprising approximately 1.3% of the total polyphenols. Anthocyanins, on the other hand, are one of the most significant phenolic compound groups found in black chokeberries (Sidor et al., 2019). These compounds are members of the flavonoid family but are characterized by their unique capability to form flavilium cations (Riaz et al., 2016). Anthocyanins in Aronia account for approximately 25% of the total polyphenol content (Jurendić and Ščetar, 2021).

Primarily, four distinct cyanidin glycosides stand out among anthocyanins: 3-O-glucoside (1.3%), 3-O-galactoside (68.9%), 3-O-xyloside (2.3%) and 3-O-arabinoside (27.5%) (Jurendić and Ščetar, 2021). Additionally, minute quantities of pelargonidin arabinoside and pelargonidin 3-O-galactoside have been identified (Oszmiański and Wojdylo, 2005). Chokeberry stands out as one of the most abundant plant sources of anthocyanins. The distinct colors of many vegetables and fruits originate from these compounds (de Pascual-Teresa et al., 2010; Minkiewicz et al., 2004; Malien-Aubert et al., 2001). Additionally, as the fruit ripens, the higher concentration of anthocyanins contributes to the enhancement of its color and visual attractiveness (Gralec et al., 2019). Environmental conditions impact the stability of anthocyanins, and consequently, their levels may decrease following sudden changes (Negreanu-Pirjol et al., 2023). The processing of fruits may significantly reduce the total anthocyanin content (Williamson, 2017). It has been observed that storing *A. melanocarpa* fruits at 70°C for 24 hours can lead to a 50% reduction in anthocyanin content (Liang et al., 2021). Phenolic acids constitute approximately 7.5% of the total polyphenolic compounds in *A. melanocarpa* extracts, where chlorogenic acid and neochlorogenic acid are the primary constituents (Oszmiański and Wojdylo, 2005). Chlorogenic acid, the principal phenolic acid, is derived from caffeic acid through an ester bond with quinic acid (Jurikova et al., 2017). Furthermore, research indicates that approximately 40% of chokeberry's antioxidant activity can be attributed to proanthocyanidins (Denev et al., 2019).

Table 3. Phenolic compounds of Aronia berries, juice and pomace

Berries	Juice	Pomace
	Polyphenols (total)	
1845–2340 mg GAE/100 g [Ochmian et al. 2012], 7.78–12.85 g GAE/kg [Rop et al. 2010], 13.3 g GAE/kg [Kapci et al. 2013], 15.0–17.9 g CE/kg [Mayer-Miebach et al. 2012], 603 mg GAE/100 g [Dudonné et al. 2015], 1079–1921 mg GAE/100 g [Wangensteen et al. 2014], 1540.01 mg GAE/100 g [Najda et al. 2013], 10637.20 mg GAE/kg; [Jakobek et al. 2007], 8563.8–12055.7 mg GAE/kg [Jakobek et al. 2012b]	11.24 g/L [Daskalova et al. 2019], 7.77–11.24 g/L [Valcheva-Kuzmanova et al. 2019], 8834–11093 mg GAE/L [Tolić et al. 2017], 3002–6639 mg GAE/L [Tolić et al. 2015], 675–755 mg /100 mL GAE [Handeland et al. 2014], 2.73–10.35 g /L GAE [Sosnowska et al. 2016], 4772.2 mg GAE/L [Daskalova et al. 2015], 4.00 g /L GAE [Pozderović et al. 2016; Popović et al. 2016], 6484 mg GAE/L [Tomić et al. 2016], 709.3 mg GAE/100 mL [Valcheva-Kuzmanova et al. 2013a], 6652 mg /L GAE [Valcheva-Kuzmanova et al. 2013b], 5461 mg GAE/L [Sainova et al. 2012], 3172–7340 mg, GAE/L [Borowska et al. 2009]	63.1 g GAE/kg [Kapci et al. 2013], 31–63 g CE/kg [Mayer-Miebach et al. 2012]
	Phenolic acids	
121.9 mg/100 g [Ochmian et al. 2009], 63.9 mg/100 g [Dudonné et al. 2015], 77–96 mg/100 g [Mattila et al. 2006]		
	Hydroxycinnamic acids	
1.4–1.5 g /kg ChAE [Mayer-Miebach et al. 2012], 116.4 mg /100 g ChAE [Wilkes et al. 2014], 739.3–1670.3 mg /kg ChAE [Jakobek et al. 2012b]	0.45–0.59 g /kg ChAE [Mayer-Miebach et al. 2012], 48.9–77.9 mg /100 g ChAE [Wilkes et al. 2014]	0.72–0.82 g ChAE/kg [Mayer-Miebach et al. 2012], 89.7–231.6 mg /100 g ChAE [Sójkaal et al. 2013]
	Anthocyanins (Total)	
256.4 mg/100 g [Vagiri et al. 2017], 6.2–6.7 g /kg CGlE [Mayer-Miebach et al. 2012], 529.3 mg/100 g [Ochmian et al. 2009], 357 mg CGlE/100 g [Dudonné et al. 2015], 249–447 mg /100 g CGaE [Wangensteen et al. 2014], 1480.0 mg CGlE/kg [Wu et al. 2004], 619.2 mg /100 g CGlE [Wilkes et al. 2014], 4056.22 mg CGlE/kg [Jakobek et al. 2007], 1500.9–5486.2 mg /kg CGlE [Jakobek et al. 2012b], 4.5 g CGlE/kg [Kapci et al. 2013], 498.98 mg/100 g [Najda et al. 2013]	0.86–2.13 g/L [Valcheva-Kuzmanova et al. 2019], 2.8–45.2 mg /100 mL CgaE, [Handeland et al. 2014], 4.76 g/L [Wilkowska et al. 2017], 221.4 mg/L [Sainova et al. 2012], 19.10 mg/100 mL [Wiczkowski et al. 2010], 1829–2768 mg CGlE/l [Tolić et al. 2017], 0.10–0.67 g CGlE/l [Sosnowska et al. 2016], 369.47 mg CGlE/l [Popović et al. 2016; Pozderović et al. 2016], 106.8 mg /100 mL CGlE [Valcheva-Kuzmanova et al. 2013a], 508–1087 mg /l CGaE [Borowska et al. 2009], 59.3–1118 mg /l CGlE [Vlachojannis et al. 2015]	738.7–1221.1, mg/100 g [Vagiri et al. 2017], 11.9–19.5 g /kg CGlE [Mayer-Miebach et al. 2012], 10 g CGlE/kg [Kapci et al. 2013]
	Cyanidin-3-O-galactoside	
417–636 mg/100 g [Skupień et al. 2007; Gralec et al. 2019; Ochmian et al. 2012], 1010.80-1203.56 mg/kg [Rop et al. 2010], 2917.2 mg CGIE/kg [Kapci et al. 2013], 4.1-4.4 g CGIE/kg [Mayer-Miebach et al. 2012], 379.36 mg/100g [Ochmian et al. 2009], 229 mg CGIE/100g [Dudonné et al. 2015], 989.7 mg CGIE/100 g [Wu et al. 2004], 1243 mg CGIE/100g [Tarko et al. 2009], 2794.74 mg CGIE/kg [Jakobek et al. 2007], 1055.3-3621.0 mg CGIE/kg [Jakobek et al. 2012b]	7.87 g/L [Oszmiański et al. 2005], 1.49 g/L [Daskalova et al. 2019], 0.64–14.98 g/L [Valcheva-Kuzmanova et al. 2019], 46.58–96.88 CGlE mg/L [Sosnowska et al. 2016], 301 mg CArE/l [Tomić et al. 2016], 3.16 g/L [Wilkowska et al. 2017], 44.0–822.1 mg CGlE/l [Vlachojannis et al. 2015], 107.6 mg/100 g [Kardum et al. 2014c], 20.0 mg/L [Valcheva-Kuzmanova et al. 2013b], 143.7 mg/L [Sainova et al. 2012]	1120 mg/100 g [Oszmiański et al. 2005], 4600.5 mg CGlE/kg [Kapci et al. 2013], 7.6–12.5 g CGlE/kg [Mayer-Miebach et al. 2012], 437.2–754.6 mg/100 g [Vagiri et al. 2017]
	Cyanidin-3-O-glucoside	
8–27 mg/100 g [Skupień et al. 2007; Gralec et al. 2019; Ochmian et al. 2012], 7.11 mg/100 g [Ochmian et al. 2009], 127 mg/kg [Kapci et al. 2013], 18.15-21.15 mg/100 g [Kader et al. 2005], 37.6 mg/100 g [Wu et al. 2004], 200.0 mg/kg [Veberic et al. 2015]	28 mg/100 g [Oszmiański et al. 2005], 0.12 g/L [Daskalova et al. 2019], 0.04–0.12 g/L [Valcheva-Kuzmanova et al. 2019], 0.3–2.0 mg CGaE/100 mL [Handeland et al. 2014], 0.16 g/L [Wilkowska et al. 2017], 4.9 mg/100 g [Kardum et al. 2014c], 4.4 mg/L [Valcheva-Kuzmanova et al. 2013a], 2.01-4.37 mg/L [Sosnowska et al. 2016], 3.7-5.7 mg/L [Bursać Kovačević et al. 2016], 9.28 mg/L [Popović et al. 2016; Pozderović et al. 2016], 21 mg/L [Tomić et al. 2016], 2.4-41.9 mg/L [Vlachojannis et al. 2015]	79 mg/100 g [Oszmiański et al. 2005], 237.7 mg/kg [Kapci et al. 2013], 33.9-52.0 mg/100 g [Vagiri et al. 2017], 0.24-0.44 g/kg [Mayer-Miebach et al. 2012]
	Cyanidin-3-O-arabinoside	
129–299 mg/100 g [Skupień et al. 2007; Gralec et al. 2019; Ochmian et al. 2012], 941.82–1553.29 mg/kg [Rop et al. 2010], 1359.4 mg CGlE/kg [Kapci et al. 2013], 1.9–2.1 g CGlE/kg [Mayer-Miebach et al. 2012], 116.39 mg/100 g [Ochmian et al. 2009], 112 mg CGlE/100 g [Dudonné et al. 2015], 52–149 mg CGaE/100 g [Wangensteen et al. 2014], 399.3 mg CGlE/100 g [Wu et al. 2004], 1243.2 mg CyE/kg [Veberic et al. 2015], 154.7 mg CGlE/100 g [Wilkes al. 2014], 146 mg CGaE/100 g [Slimestad et al. 2005], 544 mg CGlE/100 g [Tarko et al. 2009], 993.77 mg CGlE/kg [Jakobek et al. 2007], 367.2–1532.4 mg CGlE/kg [Jakobek et al. 2012b]	324 mg/100 g [Oszmiański et al. 2005], 0.50 g/L [Daskalova et al. 2019], 0.18 to 0.50 g/L [Valcheva-Kuzmanova et al. 2019];0.7–10.7 mg CGaE/100 mL [Handeland et al. 2014], 11.47–32.59 CGlE mg/L [Sosnowska et al. 2016], 78.47 mg/L [Popović et al. 2016; Pozderović et al. 2016], 1.44 g/L [Wilkowska et al. 2017], 11.3–249.9 mg CGlE/l [Vlachojannis et al. 2015], 36.2 mg/100 g [Kardum et al. 2014c], 8.2 mg/L [Valcheva-Kuzmanova et al. 2013b], 61.7 mg/L [Sainova et al. 2012], 110.1–178.7 mg /l CGaE [Borowska et al. 2009], 5.12 mg /100 mL CGaE [Krajka-Kuźniak et al. 2009]	533 mg/100 g [Oszmiański et al. 2005], 1651.1 mg CGIE/kg [Kapci et al. 2013], 3.7-5.7 g CGIE/kg [Mayer-Miebach et al. 2012], 217.5–366.7 mg/100 g [Vagiri et al. 2017]
	Cyanidin-3-O-xyloside	
53 mg/100 g [Szopa et al. 2017; Oszmiański et al. 2005], 29–38 mg/100 g [Skupień et al. 2007; Gralec et al. 2019; Ochmian et al. 2012], 165.8 mg CGIE/kg [Kapci et al. 2013], 26.40 mg/100 [Ochmian et al. 2009], 8.12 mg/100 g CGlE [Dudonné et al. 2015], 51.5 mg/100 g CGlE [Wu et al. 2004], 38.7 mg CyE/kg [Veberic et al. 2015], 9.92 mg/100 g [Tian et al. 2017], 20.1 mg /100 g CGlE [Wilkes al. 2014], 10 mg CGaE/100 g [Slimestad et al. 2005], 73 mg CGlE/100 g [Tarko et al. 2009], 146.02 mg CGlE/kg [Jakobek et al. 2007], 44.3–233.1 mg CGlE/kg [Jakobek et al. 2012b]	0.34 g/L [Oszmiański et al. 2005], 4.6 mg/L [Daskalova et al. 2019], 1.24–4.74 mg/L CGlE [Sosnowska et al. 2016], 0.2–2.1 mg /100 mL CGaE [Handeland et al. 2014], 3.2–5.2 mg/L [Bursać Kovačević et al. 2016], 6.88 mg/L [Popović et al. 2016; Pozderović et al. 2016], 0.6 mg/L [Valcheva-Kuzmanova et al. 2013b], 11.6 mg/L [Sainova et al. 2012], 0.59 mg/100 mL [Wiczkowski et al. 2010], 19.8–29.4 mg CGaE/l [Borowska et al. 2009], 0.59 CGaE/100 mL [Krajka-Kuźniak et al. 2009]	105 Pomace mg/100 g [Oszmiański et al. 2005], 223.4 mg CGlE/kg [Kapci et al. 2013], 36.7–63.3 mg CGlE/100 g [Vagiri et al. 2017], 0.3–0.6 g /kg CGlE [Mayer-Miebach et al. 2012]
	Procyanidins	
1646 mg/100 g [Ochmian et al. 2012; Gralec et al. 2019], 1426.66-1645.64 mg/100 g [Skupień et al. 2007], 663.7 mg/100 g [Wu et al. 2004], 868.6 mg CE/100 g [Wilkes et al. 2014]	15.79 g/L [Oszmiański et al. 2005], 60-72 CyE mg/100 mL [Handeland et al. 2014], 3529.1 mg CE/l [Oprea et al. 2014], 240 mg CE/l [Tomić et al. 2016], 34.2 mg CE/100 mL [Stanisavljević et al. 2015], 3122.5 mg/L [Sainova et al. 2012], 3926.2 mg/L [Valcheva-Kuzmanova et al. 2013b], 293.38 mg/100 mL [Krajka-Kuźniak et al. 2009]	8192 mg/100 g [Oszmiański et al. 2005], 24-129 g CE/kg [Hirth et al. 2015]

The Health Benefits of Chokeberry Fruits

Antioxidant Effects

Consistent intake of antioxidant-rich foods is recommended for improving overall health and reducing the risk of chronic diseases (Bal et al., 2023). Polyphenols are the primary constituents responsible for the antioxidant potential of plant-based foods, including fruits and vegetables (Ćujić et al., 2018). Polyphenols, numbering over 8000 known variants in the plant kingdom, rank as the most prevalent and crucial dietary antioxidants, significantly adding to the antioxidant potential of plant-based foods (Ćujić et al., 2018; Pavlovic et al., 2015).

Polyphenols exert antioxidant effects as a result of their redox potential. Here, acting as hydrogen donors and singlet oxygen quenchers, polyphenols have the ability to serve as reducing agents with the potential for metal-chelation. These attributes empower polyphenols to combat oxidative stress effectively, demonstrating strong safeguards against oxidative damage at the cellular level (Oszmiański and Wojdylo, 2005). Oxidative damage or oxidative stress occurs when there is an imbalance between radical formation and antioxidant defense (Ehlenfeldt et al., 2001; Moyer et al., 2002; Mohammed et al., 2023a). It is also a significant factor in the development of various diseases, such as Alzheimer's disease, stroke, heart disease, and Parkinson's disease (Denev et al., 2012; Mohammed et al., 2023b). A range of antioxidants alleviate oxidative stress and can delay or prevent the oxidation of specific substrates (Ehlenfeldt et al., 2001). Including dietary antioxidants as a component of a balanced diet is advised to bolster the body's antioxidant defenses and reduce the risk of cardiovascular and other chronic diseases (Arts and Hollman, 2005; Hooper et al., 2008; Uysal et al., 2023). Chokeberry is considered to be one of the most potent dietary antioxidants due to its substantial polyphenol content (Ćujić et al., 2018).

Previous research has shown that chokeberry has significantly higher antioxidant potential compared to other fruits, such as cranberries, blueberries, and black currants (Ehlenfeldt et al., 2001; Denev et al., 2012). Evaluating the overall phenolic content and antioxidant capacity of various fruits, chokeberry has been found to exhibit substantially higher total antioxidant capacity when compared to other fruits like cranberries, blueberries, and black currants. Black chokeberry boasts a wealth of polyphenols, vitamins C and E, and essential minerals like copper and zinc (Zhang et al., 2021). These constituents contribute to the heightened antioxidant capacity of chokeberry (Seeram et al., 2008). Chokeberry's antioxidant activity has also been demonstrated through *in vitro* studies. *In vitro* antioxidant activity of Aronia berries has been attributed to approximately 40% proanthocyanidins, followed by anthocyanins (24%), hydroxycinnamic acids (18%), and epicatechin (11%) (Jurendić and Ščetar, 2021).

The *in vivo* mechanisms of polyphenol antioxidant activity surpass radical scavenging and encompass functions such as reducing reactive nitrogen and oxygen species post-absorption, restoring antioxidant enzymes, inhibiting prooxidants, and modulating cellular signaling related to antioxidant levels and enzymes (Pavlovic et al., 2015; Tolić et al., 2015; Unal et al., 2022). For example, a study investigating the influence of chokeberry extract on oxidative stress in human plasma treated with homocysteine showed that the introduction of chokeberry extract led to a rise in overall antioxidant capacity (Malinowska et al., 2012). Kedzierska et al. (2012) showcased the suppressive effects of chokeberry extract on the generation of superoxide anion radicals in platelets collected from both breast cancer patients and healthy individuals. Chokeberry anthocyanin extract exhibited protective effects against hydrogen peroxide and high glucose-induced oxidative stress and cytotoxicity in pancreatic β-cells (βTC3) (Ćujić et al., 2018). Moreover, pre-treatment of pancreatic β-cells with chokeberry extract resulted in increased activity of antioxidant enzymes comparison to cells solely exposed to prooxidant agents (Rugină et al., 2015). In another study, the antioxidant activity was examined during a clinical trial involving 11 healthy volunteers who consumed 250 mL of Aronia fruit juice daily for three weeks. The participants' serum antioxidant capacity, tested using spectrophotometric methods with stable DPPH radical cations, showed a significant increase (Nowak et al., 2016). Black chokeberry juice has been found to have cardioprotective effects and can slow down atherosclerosis, as well as possessing anti-aging properties (Zhang et al., 2021). According to Pilaczynska-Szczesniak et al. (2005), rowers who consumed 150 mL of fruit juice daily during a one-month training camp experienced a decrease in oxidative damage to red blood cells induced by exercise. Green, unripe chokeberry fruits lack anthocyanins but exhibit high antioxidant activity due to their elevated proanthocyanidin and flavonoid content (Gralec et al., 2019). Results obtained by Szopa et al. (2017) demonstrated that leaves of Aronia species also exhibit robust antioxidant capacity, making them potentially interesting for therapeutic and dietary purposes. Additionally, apart from chokeberry fruits, fruit products, and post-production waste also demonstrate antioxidant potential (Sidor and Gramza-Michałowska, 2019). Nawirska et al. (2007) reported that chokeberry pomace showed moderate activity when compared to other pomaces. On the other hand based on Pieszka et al. (2015)'s findings, dried chokeberry pomace demonstrated superior antioxidant properties compared to apple, black currant, carrot pomaces, and strawberry. Najda and Łabuda (2013) conducted research on various fruits' antioxidant activity and observed that chokeberry fruits exhibited greater antioxidant activity compared to the other eight fruits. Analyzing the antiradical activity of fruit juice and pomace (fruit residue), Sidor and Gramza-Michałowska (2019) showed that pomace exhibited the highest activity, followed by fruit and fruit juice. In testing the antioxidant potential of fruit juices, chokeberry juice demonstrated the highest DPPH radical scavenging ability (72.44 mol TE/mL) (Jakobek et al., 2007). Keskin-Šašić et al. (2012) confirmed that Aronia juice demonstrated higher antiradical activity than 14 other fruit juices. Zheng and Wang (2003) evaluated the antioxidant activity of chokeberry in a study excluding proanthocyanidins and found that activity was mainly derived from anthocyanins

(53.1%), phenolic acids (38.2%), and flavonols (8.7%). Research on chokeberry products has revealed that the antioxidant potential of these products varies based on the harvest time and the year of the raw material (Tolić et al., 2017; Bolling et al., 2015). Another crucial factor affecting the antioxidant properties of Aronia products is the technological production processes (Sidor and Gramza-Michałowska, 2019).

Antioxidant compounds play a vital role in promoting human health by preventing damage to normal cells through their ability to counteract and inhibit free radicals. Their antioxidant effects are valuable in alleviating conditions caused by oxidative stress, such as cancer, infections, heart disease, and diabetes (Ren et al., 2022; Mohammed et al., 2022). In this context, the significant abundance of phenolic compounds and other natural substances exhibiting robust antioxidant activity in Aronia berries might contribute to enhancing human health (Sun et al., 2017).

Effects on Cardiovascular Health

One of the prevalent chronic conditions is cardiovascular disease, and its share in global mortality and morbidity is considerable. Metabolic disorders associated with oxidative stress, hypertension, and obesity are among the risk indicators for cardiovascular diseases. Epidemiological investigations carried out in both industrialized and developing nations have confirmed that hypertension contributes the most to the development of cardiovascular and related diseases (Catalán-Ramos et al., 2014; Mancia et al., 2007). Regular consumption of chokeberry and its products can mitigate numerous risk factors that trigger the onset of cardiovascular diseases.

In a study, Chokeberry juice led to a significant reduction in diastolic blood pressure after six weeks of regular intake in men with mild hypercholesterolemia (Skoczynska et al., 2007). A comprehensive analysis of controlled clinical trials indicated that daily supplementation with Aronia fruit extract for six to eight weeks significantly lowered systolic blood pressure and overall cholesterol levels, which are key factors in cardiovascular disease risk, in adult participants (Hawkins et al., 2021). In another clinical research study, 23 patients without pharmacologically treated grade I hypertension consumed 200 mL of phenolic-rich Aronia juice daily for four weeks, resulting in a significant decrease in average 24-hour, awake systolic and diastolic blood pressure. Additionally, triglyceride levels and total low-density lipoprotein cholesterol levels exhibited notable reductions after four weeks of Aronia juice consumption (Kardum et al., 2015).

The favorable impact of consuming Chokeberry juice and extract on blood pressure is likely attributed to the substantial presence of phenolic compounds within them. Polyphenols can influence hypertension and general cardiovascular well-being through their capacity to diminish oxidative stress within the blood vessels (Jurendić and Ščetar, 2021).

In a study involving 44 patients with a previous heart attack, a double-blind, placebo-controlled, parallel investigation was conducted, where black chokeberry extract was administered via injection, and its impacts were assessed. Participants who were administered the extract for a duration of 6 weeks exhibited notable reductions in LDL levels, inflammation, and oxidative stress in comparison to individuals solely consuming statin medications (Valcheva-Kuzmanova et al., 2005). Studies conducted on rats with induced hypertension showed that the treatment group treated with *Aronia melanocarpa* ethanolic extract exhibited lower blood pressure values compared to the control group. The decrease in blood pressure was emphasized to be associated with enhancement of overall antioxidant capacity and mitigation of lipid peroxidation (Ciocoiu et al., 2013). Both *in vivo* and *in vitro* tests indicate that phenolic compounds play a role in safeguarding and rejuvenating endothelial cells (Poreba et al., 2009) and have antiplatelet effects (Bijak et al., 2011; Olas et al., 2008). Given that oxidative stress might play a role in the development of cardiovascular diseases, it is suggested to consume dietary antioxidants such as polyphenols as a means of preventing them. Another significant health effect of polyphenols is their role in regulating lipid levels. Due to their antioxidant effects, polyphenols can protect lipids against oxidation in both food items and the body. This is particularly important for foods rich in unsaturated fatty acids that are prone to lipid peroxidation, since the byproducts of lipid peroxidation can enter the body and result in harm (Ćujić et al., 2018).

Polyphenols found in the gastrointestinal system can improve lipid oxidation levels and reduce the adverse effects of lipid peroxidation products (Görelik et al., 2013; Kanner et al., 2012). Additionally, certain polyphenolic compounds can impact the production of lipids in the liver and/or their processing in the intestines. Therefore, through a range of mechanisms, polyphenols sourced from foods like chokeberry can positively impact lipid levels (Ćujić et al., 2018). In a study, six weeks of chokeberry juice supplementation resulted in a significant decrease in levels of triglycerides, overall cholesterol, and LDL cholesterol among men with mild hypercholesterolemia (Poreba et al., 2009). Another study found that four weeks of chokeberry fruit juice consumption lowered triglyceride levels in individuals with high-normal blood pressure or grade I hypertension (Kardum et al., 2015). In another study, the intake of 300 mg of chokeberry extract daily for two months significantly reduced triglycerides, total cholesterol, and LDL cholesterol levels in subjects with metabolic syndrome (Broncel et al., 2010). Additionally, out of the 31 fruit extracts examined, chokeberry exhibited the highest efficacy in inhibiting pancreatic lipase activity. As pancreatic lipase plays a pivotal role in the assimilation of dietary triglycerides, these results imply that incorporating chokeberry fruits into the diet could serve as a strategy to diminish fat absorption (Sosnowska et al., 2015).

In a parallel and placebo-controlled study conducted by Stojković et al. (2021), the effects of daily consumption of Aronia juice for four weeks on peripheral blood leukocytes' nucleotide element-1 DNA methylation and plasma profiles of polyunsaturated fatty acids (PUFAs) were examined in individuals at risk for cardiovascular disease. The outcomes indicated that the ingestion of Aronia fruit led to a decrease in LINE-1 methylation levels and the ratio of arachidonic acid to eicosapentaenoic acid. Given the connection between cardiovascular disease, DNA methylation, and alterations in PUFA profiles, this clinical

investigation showcased potential cardiovascular protection through consistent consumption of Aronia juice.

Disturbed glucose metabolism is another risk factor in the development of cardiovascular diseases. Some findings indicate that chokeberry may have a positive effect on blood sugar levels. Bräunlich et al. (2013) proposed that distinct phenolic components found in chokeberry have the potential to act as effective inhibitors of α-glucosidase, thereby lowering blood sugar levels and potentially preventing the onset of diabetes.

Indeed, clinical trials have evaluated the benefits of two main phenolic components of Aronia berries, namely chlorogenic acid and quercetin, for the purpose of averting and managing cardiovascular ailments. There are studies examining the impact of a natural supplement comprising luteolin and chlorogenic acid on cardio-metabolic risk factors in individuals diagnosed with metabolic syndrome, as well as clinical trials investigating the impact of chlorogenic acids on the human vascular system. These studies aim to shed light on the potential cardioprotective effects of these compounds.

Clinical studies demonstrate that the active constituents with possible impacts to reduce the risk of cardiovascular disease are the potent phenolic compounds found in Aronia fruits, known for their strong antioxidant activity. Both chlorogenic acid and quercetin, among these phenolic compounds, could be considered as promising lead compounds for the development of novel substances for the management and prevention of cardiovascular disorders (Ren et al., 2022).

Anti-inflammatory effects

The Aronia berries is effective in preventing the development of chronic diseases because of its ability to reduce inflammation. Ohgami et al. (2005) demonstrated the anti-inflammatory effects of extract from *Aronia melanocarpa* on uveitis (It is the inflammation of a part or all of the uvea layer in the eye. It is an inflammatory disease.), an endotoxin-induced inflammatory disease, in rats. In another study, the ingestion of 100% cold-pressed fruit juice and oven-dried black chokeberry powder among individuals with slightly elevated blood pressure did not lead to significant alterations in the majority of inflammatory markers, but it was reported to decrease IL-10 (Interleukin 10 is classified as an anti-inflammatory cytokine and is alternatively referred to as a human cytokine synthesis inhibitory factor. It negatively regulates the immune response to microbe-derived antigens) and TNFa (or Necrosis Factor alpha is a cellular signaling protein implicated in systemic inflammation, and it's among the cytokines contributing to the acute phase reaction.) levels (Loo et al., 2016). Sikora et al. (2014a, 2014b) examined the impact of extract from black chokeberry on individuals diagnosed with metabolic syndrome and reported a slight increase in CRP (C-Reactive Protein is a blood examination that gauges inflammation within the body, irrespective of whether one is in a fasting or non-fasting state. It indicates the presence and degree of inflammation.) levels, which, however, did not decrease significantly following a period of two months consuming black chokeberry. Cyclooxygenases and inducible nitric oxide synthase are pivotal enzymes with proinflammatory roles, accountable for generating lipid mediators and nitric oxide, respectively, which are associated with the progression of many inflammatory diseases (Li et al., 2017). Jang et al. (2020) demonstrated that the Aronia bioactive fraction inhibits the production of COX-2 and iNOS in airway epithelial cells and reduces the secretion of reactive oxygen species, inducing cell cycle arrest and providing clear evidence for its anti-inflammatory activity.

Antidiabetic effects

Aronia fruits exhibit potential antidiabetic activity due to their ability to combat oxidative stress triggered by hyperglycemia (Banjari et al., 2017). Research has indicated that black chokeberry can positively impact β cells by shielding them from the detrimental consequences of oxidative stress (Sidor and Gramza-Michałowska, 2019). According to Rugină et al. (2015), the anthocyanin component of black chokeberry curtailed the harmful impact of glucose on pancreatic β cells (TC-3) in mice, leading to an enhancement in β cell viability. The extract from black chokeberry resulted in a noteworthy decrease in the concentration of reactive oxygen species in H_2O_2-exposed β cells. In a diabetic animal model studied by Mu et al. (2020), male Wistar rats (approximately 200 g) were given Aronia fruit ethanol extract (100 mg/kg) for eight weeks, resulting in significant reductions in blood glucose and serum insulin levels, insulin resistance degrees, and improved glucose tolerance level and hepatic glycogen.

Phenolic compounds derived from natural sources play a role in regulating carbohydrate and lipid metabolism, as well as blood sugar levels. They also mitigate insulin resistance, oxidative stress, and inflammation. Consequently, they have garnered widespread attention for their potential in managing and preventing diabetes (Dragan et al., 2015; Chen et al., 2021). For instance, ellagic acid derived from Aronia berries has been found to reduce hepatic oxidative stress and insulin resistance in a type 2 diabetic animal model. When ellagic acid (50 mg/kg) was administered daily to 11-month-old female Goto-Kakizaki rats for 28 days, blood sugar and insulin resistance were significantly reduced (Park et al., 2013). This points to the compound's antidiabetic effect for the treatment of hepatic complications in type 2 diabetes (Polce et al., 2018).

Monosaccharides like glucose and fructose are taken up by the small intestine, whereas disaccharides and polysaccharides are converted into monosaccharides within the intestine through enzymatic processes involving α-glucosidase and pancreatic α-amylase. Inhibition of α-glucosidase leads to reduced carbohydrate breakdown, altering the digestive process to occur in the latter portion of the small intestine, consequently decreasing the absorption of glucose into the bloodstream. This approach is regarded as a promising tactic to decrease levels of glucose in the blood and potentially alleviate complications associated with diabetes (Zhang et al., 2021). The chemical constituents found in the leaves of black chokeberry exhibit strong antioxidant characteristics and are capable of inhibiting the activities of α-amylase and α-glucosidase. This suggests significant potential for their use in potential treatments for both Alzheimer's disease (AD) and type 2 diabetes. (Zdunić et al., 2020). Research indicates that the consumption of chokeberry juice can lead to a decrease in

glucose levels following an oral glucose tolerance test. Furthermore, it can notably diminish the activities of dipeptidyl peptidase IV, α-glucosidase, and angiotensin-converting enzyme in a manner that correlates with the dosage administered (Kumar et al., 2011). In a separate investigation, 35 individuals diagnosed with type 2 diabetes integrated phenol-rich Aronia juice (150 mL thrice daily, 50 mL per intake) into their conventional diabetes treatment. The patients displayed enhanced health conditions, suggesting that Aronia juice holds potential as a promising approach for diabetes mellitus prevention and management (Milutinović et al., 2019).

Support can be provided for treating diabetes or preventing metabolic syndrome by agents that aid in the absorption of carbohydrates and fats in the digestive system. It has been found that black chokeberry polyphenols also inhibit lipase activity, such as glucosidase (Sidor et al., 2019). Studies conducted on rats fed a high-fructose diet and treated with black chokeberry extract have shown that it lowered their blood glucose levels and improved lipid profiles (Qin and Anderson, 2012). Other research has demonstrated that black chokeberry extract lowers glucose levels in rats induced with hyperglycemia, obesity, and high-fat diets (Takahashi et al., 2015), as well as insulin-resistant mice treated with black chokeberry extract administered at levels of 100 and 200 µg/kg over a span of eight weeks (Park and Park, 2011). Yamane et al. (2017) noted that consuming chokeberry juice before meals reduced postprandial blood sugar levels. However, some other studies on black chokeberry products have shown no significant change in glucose levels in obese, hypertensive, or healthy individuals (Kardum et al., 2014a; Kardum et al., 2014b; Kardum et al., 2014c; Kardum et al., 2015; Loo et al., 2016).

Lipińska and Jóźwik (2018) demonstrated the ability of black chokeberry pomace to induce hypoglycemic effects in Merino lambs. In the control group, the glucose level measured 3.38 mmol/L, whereas the experimental groups that received 150 and 300 g of black chokeberry per kilogram of feed exhibited glucose levels of 2.42 and 1.55 mmol/L, respectively. Previous studies have indicated that the ample presence of niacin and anthocyanins in chokeberries contributes to their beneficial effects. Patients with hypercholesterolemia treated with daily chokeberry juice for 6 weeks showed a decrease in low-density lipoprotein cholesterol, triglyceride levels, and total cholesterol, as well as an increase in high-density lipoprotein cholesterol levels. Chokeberry juice assisted in their improvements without any pharmacological treatment (Valcheva-Kuzmanova et al., 2007).

High-fat diet-induced liver damage is closely associated with inflammation. Cyanidin-3-O-galactoside from black chokeberry has been shown to alleviate liver damage and inhibit the secretion of pro-inflammatory factors caused by a high-fat diet (Jiao et al., 2021). Individuals with diabetes who consumed 200 mL of black chokeberry juice on a daily basis for a period of three months observed a notable decrease in fasting blood glucose levels (Pinent et al., 2004); beneficial effects of black chokeberry juice on cholesterol and HbA1c-glycosylated hemoglobin lipid levels have been observed (Bell et al., 2006).

As summarized above, *Aronia* fruits have the potential to exhibit anti-type 1 diabetes and anti-type 2 diabetes activities, primarily due to the contribution of their existing phenolic compounds. These substances exert their antidiabetic effects by alleviating the oxidative stress induced by elevated blood sugar levels (Ren et al., 2022).

Antimicrobial effects

Much like their antioxidative properties, the antibacterial efficacy of plant extracts originates from the existence of phenolic compounds (Staszowska-Karkut and Materska, 2020). In fruit crops, polyphenols serve as remarkably efficient antimicrobial agents. The synthesis of flavonoids in plants is recognized as a defensive reaction to microbial infections; therefore, their *in vitro* effectiveness against a wide range of microorganisms is not surprising (Jurikova et al., 2017). Kim et al. (2020) reported that Chokeberry extracts showed *in vitro* bacteriostatic activity against *Escherichia coli* and *Staphylococcus aureus*, while leaf extracts exhibited inhibitory effects on the growth of Bacillus cereus. An examination of microbial activity against ten distinct pathogens demonstrated that proanthocyanidins exhibited the highest efficacy as antimicrobial agents (Pavlovic et al., 2015). Tian et al. (2018) indicated a direct connection between the overall phenolic content and the antibacterial effects against *Staphylococcus aureus* and *Bacillus cereus*. They emphasized that ellagitannins and isorhamnetin di- and tri-glycosides are the primary inhibitors, while the composition profiles significantly influence the antibacterial potential of plant extracts. According to Alves et al. (2013), the effectiveness of phenolic acids as antibacterial agents primarily hinges on the existence of carboxyl groups and the arrangement of substitutions on the benzene ring. Certain researchers have demonstrated that the quantity of hydroxyl groups within molecules can impact the antimicrobial potential of phenolic compounds, and the addition of sugar molecules to flavonols can diminish their efficacy in inhibiting growth (Puupponen-Pimiä et al., 2001; Rauha et al., 2000).

In a study conducted by Cvetanović et al. (2018), the antimicrobial effects of extracts derived from Aronia leaves were examined against two types of gram-positive and four types of gram-negative bacterial strains, and the extract's ability to combat fungal activity against two fungal species was also evaluated. The researchers conducted a comparison between the antimicrobial efficacy of Aronia extracts and amracin (a tetracycline antibiotic). They observed that the leaf extracts demonstrated antibacterial effectiveness that was fourfold greater against P. vulgaris and fifteenfold stronger against Proteus mirabilis in comparison to amracin. In another study, Chojnacka et al. (2020) demonstrated that *Aronia melanocarpa* fruit juice inhibited the replication of influenza viruses in their early stages and exhibited *in vitro* and *in vivo* effectiveness against various subtypes of influenza viruses. The ability to combat influenza was attributed to two polyphenolic compounds, namely myricetin and ellagic acid. Lately, there has been an observation that diverse plant species harbor biologically active substances, notably polyphenols. These compounds exhibit efficacy in addressing a range of illnesses, especially when combined synergistically, and function as natural inhibitors of viral enzymes. Besides other phytochemicals, ellagic acid and quercetin have shown potential antiviral activity against SARS-CoV-2 when interacting with viral proteins (Zhang et al., 2020).

Anticancer effects

Chokeberry, like other fruits, is an excellent reservoir of polyphenolic compounds with anti-tumor potential in both animals and humans. The antitumor activity is primarily associated with chlorogenic acids, certain cyanidin glycosides, and quercetin derivatives (Jurendić and Ščetar, 2021). In one study, the antioxidative capacity of Aronia berries was found to be associated with their anthocyanin content and total proanthocyanidin, and the present cyanidin glycosides were shown to inhibit cancer cell proliferation (Rugină et al., 2012). Commercial extracts of red, purple, and black chokeberries underwent analysis for their overall phenolic content and antioxidants, and only the black chokeberry extract was found to be active against HT-29 cells; this activity was shown to be related to the overall phenolic composition, antioxidative potential, and quantities of caffeic and chlorogenic acids (Gill et al., 2021).

In recent times, research has been undertaken to explore chokeberry's defensive mechanisms against cancer and its anti-cancer activity on tumor cell lines (Zhao et al., 2004; Bermúdez-Soto et al., 2007). Black chokeberry has been reported to have the potential to prevent the development of colon cancer, leukemia, and breast cancer, and there are also documented accounts of its preferential influence on cancer stem cells (Sidor et al., 2019). A recent examination explored the impact of black chokeberry fruit juice on mouse embryonic cancer stem cells (P19) and revealed its ability to impede the process of carcinogenesis (Sharif et al., 2013). Chokeberry juice can serve as a raw material for the production of various polyphenolic compounds, including those with antitumor activity (Sidor et al., 2019). Moreover, there are reports suggesting that Aronia leaf extract demonstrates anti-cancer effects by restraining the growth of SK-Hep1 human hepatoma cells and preventing the metastasis of cancer cells (Hwang et al., 2018).

Oxidative stress is among the indications of cancer. It has the potential to negatively impact the progression of the illness and contribute to the emergence of additional health disorders. Anticancer medications elevate oxidative stress within platelets and interfere with their physiological functions. Studies have shown that black chokeberry extract exhibits radical-scavenging inhibitor effects on platelets obtained from individuals diagnosed with breast cancer (Sidor et al., 2019). In the studies conducted by Kędzierska et al. (2013a, 2013b), *in vitro* experiments involving black chokeberry extract revealed a reduction in the levels of superoxide anion radicals in platelets obtained from all female groups that were tested. The extract was able to cleanse the radicals in platelets of diseased women by 8.7% to 35.0% and in platelets of healthy women by up to 60.7%.

It is believed that the effect of Aronia extract on platelets is due to its antioxidant activity, which is mediated through the scavenging of free radicals by polyphenols. Furthermore, it has been suggested that polyphenols increase the activity of superoxide dismutase, an important enzyme in regulating platelet function, thereby enhancing the endogenous antioxidant capacity (Sidor et al., 2019). In a study by Gao et al., (2018), it was shown that phenolic compounds found in Aronia fruits robust antioxidant potential and cytotoxic effects against HepG2 human liver cancer cells, indicating the potential of chokeberry in preventing the progression of liver tumors.

Cvetanović et al. (2018) discovered that black chokeberry extracts exhibited pronounced sensitivity towards human colorectal adenocarcinoma LS-174T cells, with IC50 values of 5.44 µg/ml for fruit extract and 1.38 µg/ml for leaf extract, compared to 7.46 µg/ml for the anticancer drug cisplatin. Additionally, the fruit extract showed cytotoxicity against HeLa cervical cancer cells, and this effect was even higher than its effect on human colorectal adenocarcinoma LS-174T cells and human A-549 lung cancer cell lines. In their study, Cvetanović et al. (2018) found that the leaf extract more strongly restrained the growth of cancer cells compared to cisplatin and fruit extract.

It is recognized that cigarette smoke harbors elevated concentrations of cancer-causing compounds. Studies have demonstrated that short-term exposure of mice to cigarette smoke resulted in a range of unfavorable alterations, including weight loss, micronucleated erythrocytes, and histopathological changes in the lungs, liver, and bladder (Sidor et al., 2019). Balansky et al. (2012) documented that water-based solutions of black chokeberry and strawberry extracts restricted the development of pulmonary emphysema, lung adenomas, and liver degeneration induced by cigarette smoke. As discussed above, Aronia fruits and their components, especially the main phenolic compounds found in the findings from clinical trials, suggest that Aronia fruits hold promising potential for the creation of novel anticancer agents.

Conclusions and Suggestions

Aronia (*A. melanocarpa*) possesses numerous positive pharmacological activities that could exert beneficial impacts on human well-being. Chokeberry fruit and its products serve as a source of various compounds that promote health and offer beneficial effects (Zhang et al., 2021). The main components of *A. melanocarpa* related to nutrition and health include polyphenols, sugars, minerals, and vitamins. Multiple *in vitro* and *in vivo* research have affirmed that these compounds showcase a diverse array of advantageous and physiological effects, including antioxidant, antidiabetic, anti-inflammatory, blood pressure-lowering, antiviral, anticancer, antiplatelet, and antiatherosclerotic activities (Jurendić and Ščetar, 2021).

As previously discussed and highlighted in this review, *Aronia* fruits demonstrate potent antioxidant activity and possess the capacity to hinder the activity of various radical species through distinct mechanisms. Therefore, these compounds hold promise in terms of preventing and potentially managing conditions such as cancer, cardiovascular diseases, diabetes, obesity, and neurological disorders (Sidor et al., 2019; Yang et al., 2021; Jurendić and Ščetar, 2021; Kokotkiewicz et al., 2010; Sun et al., 2017). *Aronia* has also found applications in addressing different types of cancers, including breast cancer, intestinal cancer, and leukemia. The therapeutic impacts of chokeberry hold significance in managing various human ailments, encompassing conditions such as hyperlipidemia, hypertension, hypercholesterolemia, diabetes, and cardiovascular diseases. Aronia berries can provide an essential strategy for enhancing human health. Chokeberry stands out as one of the most abundant sources of consumable polyphenols (Zhang et al., 2021).

Polyphenols have been documented to have beneficial health effects, particularly in terms of antioxidant potential. Besides polyphenols, the biological effects are closely linked to other prominent constituents present in chokeberries, like anthocyanins, proanthocyanidins, flavan-3-ols, and flavonol glycosides, which play a pivotal role in conferring antioxidant capabilities (Ćujić et al., 2018). Numerous literature reports on Aronia fruits have highlighted that their biological characteristics stem from the combined action of all phenolic compounds rather than isolated individual components. This presents novel avenues for research in this domain due to the diverse array of phenolic compounds present in the fruits (Negreanu-Pirjol et al., 2023).

Currently, chokeberry products available in the market mainly include fresh and dried fruits, fruit juices, jams, and jellies (Ćujić et al., 2018). Studies indicate that chokeberry possesses considerable nutritional value, and its derivatives hold substantial prospects for development (Zhang et al., 2021). However, a problem related to chokeberry polyphenols is their low stability, as none of the mentioned products provide resistance against long-term stability, oxygen, light, and moisture. Promisingly, new microencapsulation techniques may offer a solution to overcome the issues of polyphenol instability, low bioavailability, and deterioration (Ćujić et al., 2018).

However, current research tends to focus in the context of preventing and managing human illnesses, overlooking the investigation of biological toxicity, optimal consumption of polyphenols, and potential adverse effects of excessive intake such as tannins present in chokeberry. Therefore, in the future, there is a need to explore the effects and safety mechanisms of chokeberry on humans. It is anticipated that the future will witness a rise in the widespread acceptance of chokeberry-based food products, leading to an expanded consumer inclination towards functional foods (Zhang et al., 2021).

Recognizing the significance of *Aronia melanocarpa* items and residuals in human dietary intake, along with their contributions to human health, holds substantial significance. Existing literature provides data indicating the potential of *Aronia melanocarpa* as a nutrient-rich and healthy dietary food with numerous functions and benefits (Jurendić and Ščetar, 2021). The polyphenols found in Aronia are recognized as paramount dietary antioxidants, manifesting robust safeguards against cellular oxidative harm and counteracting oxidative stress both directly and indirectly (Ćujić et al., 2018).

Comprehensive research involving human studies is needed to understand the effectiveness, safety, mechanisms of action, activity, and interactions of chokeberry with other compounds, as well as to determine the recommended intake.

Competing Interests

The author declare that have no competing interests.

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