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Int J Agric Environ Food Sci 6 (1), March 2022

# Contents

## Research Articles

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Int J Agric Environ Food Sci 6 (1), March 2022

# Contents

## Research Articles

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Research Article
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# Poultry manure effects on yield and some agronomic components of Soybean (*Glycine max* L.) under Khost agro-ecological conditions, Afghanistan

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## Abstract

Soybean [*Glycine max* (L.) Merrill], which has the highest protein content of all food crops, is the world's leading source of protein and oil. Soybeans are commonly consumed by humans in the form of soymilk, soy protein, tofu, infant formula, miso, natto, soy flour, and soy sauce. They are a popular protein-rich food source in most Asian countries. Worldwide, approximately 85% of soybean's produce has been processed into soy food. While in developing countries like Afghanistan, limited farmers used inorganic fertilizers in soybean fields through the high cost, marketing problems and poor economic conditions. If we have alternative resources for soil fertility like organic manure, this problem can somewhat be solved. The objective of this study was to test the effects of different levels of poultry manure on the yield and yield components of the soybean crop. The field experiment was conducted in 2019 in Almara Village of Nadar Shah Kot District of Khost Province. Soybean variety LD04-13265 was grown with four levels of manure (0, 1, 2, and 4 tons $ha^{-1}$) using Randomized Complete Block Design with three replicates. Results revealed that manure invariably influenced most parameters under study. The highest grain yield (1212.95 kg $ha^{-1}$) was obtained with 4 tons $ha^{-1}$ (T3) followed by T2 and T1 (1145.16 and 1138.24, respectively). Poultry manure also had positive effects on the agronomic characters. Plant height, number of branches, pods $plant^{-1}$, seed $pod^{-1}$ were among the most affected. Correlation analysis revealed significant positive correlations among the grain yield and yield components but non correlation was found for 100 seed weight with other observed parameters. As per the results of this research, 4 tons of poultry manure is the optimum amount to be used for soybean production.

## Keywords

Soybean, Poultry manure, Yield and Yield components

## Introduction

Soybean [*Glycine max* (L.) Merill] is a summer annual plant that belongs to the *Leguminosae* family originated from Eastern Asia (Berk, 1992). It is a protein-rich crop with other nutrients essential for human health (Dalanin, 2015). Soybean's protein is alike to animal's protein such as meat, milk, and eggs and its leftovers are good for livestock feeding (Sopha, 2017). In 2003, Nutrition Education International (NEI) introduced soybeans to the Agriculture system of Afghanistan to save the children life because undernourishment contributed to high newborn and maternal death rates in Afghanistan (NEI, Afghanisatn), unfortunately insecure conditions and weak extension program through the government caused the less area of cultivation with low yield. On the other hand the high cost of inorganic fertilizer, marketing problems and poor economic condition can cause limited farmers in Afghanistan to use inorganic fertilizers in their fields. Tesfa et al. (2001) observed that the use of inorganic fertilizers in developing countries is insignificant as most of the smallholder farmers cannot afford even a single bag to apply to their crops. Moreover, increase in the prices of chemical fertilizers, lack of consistency in feeding the soil and endangering human health caused the increase of the use of organic manure for soil fertility (Mokhtariniya and Siadat, 2011). Demand for sustainable agriculture is to use natural resources for sustain crop production, soil productivity and for a better environment. In intensive cropping systems the

continuous use of inorganic fertilizers leads to increased soil acidity and nutrient imbalance which adversely affects soil health because of their susceptibility to losses through gaseous form and by leaching (Amoah *et al.*, 2012).

As we know, there are many factors for high yield of soybean such as soil fertility, agronomic practices, genetically improved variety, pest control and so on. Organic fertilizers like farm manure, poultry manure, compost, green manure and other are the substances that contribute to soil fertility and these types of resources are available for farmers in many areas. Plant Nitrogen needs are met by soil Nitrogen as well as chemical fertlizer. Through building soil organic amendment and mineralizable soil Nitrogen, crop production is less dependent on Nitrogen additions resulting in more sustainable agro - ecosystems (Spargo et al., 2011). Parallel soybean crop can enhance soil fertility and productivity through nitrogen fixation by symbiotic bacteria (Almaz, 2017). Poultry manure in addition to provid essential neutrients for soil fertility, it augment the biological activity of microorganisms for mineralization of compound substances (Blair et al., 2014).

The use of poultry manure can help in soil with water holding capacity, soil aeration and improved soil structure that causes the high yield of soybean (Passos, 2014). Investigation by Baghadai et al. (2018) indicated that there was no difference among the NPK fertilizer and poultry manure who tried on soybean within silage corn intercrop system.

Therefore, the current research was conducted to determine the effects of different levels of poultry manure on yield and some agronomic components of soybean under the Khost agro – ecological conditions.

## Materials and Method

### Study area

The field experiment was carried out in Almara Village, Nadar shah kot District, Khost, Afghanistan (altitude of 1386 m above sea level) during the 2019 growing season, Fig 1. Soil of the experimental site was sandy loam with 7.7 pH degree. Metrological data (Temperature and rainfall) was recorded for each month, Figure 2.

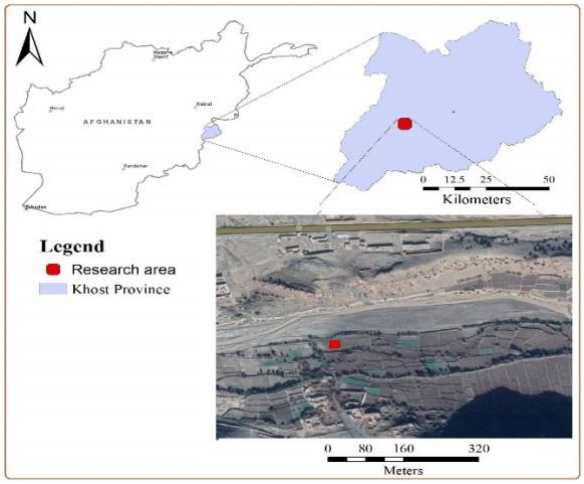


Figure 1. Map and View of study area

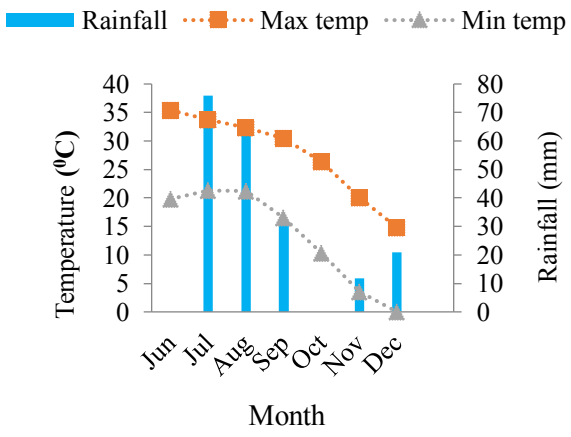


Figure 2. Climatic chart for growing season (Jun – Dec. 2019)

### Treatments and experimental design

Four levels of poultry manure (T0= 0 ton $ha^{-1}$, T1= 1 $ton^{-1}$, T2= 2 ton $ha^{-1}$ and T3= 4 ton $ha^{-1}$) were used as treatments to determine the effects on soybean variety (LD04- 13265 USA). The experimental design was Randomized Complete Block with three replications.

### Sowing

The Soil for the experiment was plowed with plough about 20- 25 cm with poultry manure to mix with soil for immediate decomposition before planting. The individual plot size was 2m x 3m ($6m^2$). The soybean crop was grown by hand on both ridges of the furrow, space between the ridges was 40 cm and apart from the crop was 20 cm. All the other agriculture practices are done according to the need of the plant during the growing season.

### Data Collection Methodology

From each plot five crops were randomly selected and tagged, then the data for the following parameters were measured.

**Plant height (cm):** Plant height was measured from the surface of soil to the top at the full pod stage.

**Number of branches**: Number of branches were recorded also in the Full pod stage.

**Number of Pods $plant^{-1}$:** After the harvest, total pods from the five plants were collected then the mean was recorded for each plot.

**Number of seeds $pod^{-1}$**: From the above sample, after the threshing number of seeds were divided on the number of pods then the mean was observed.

**100 seeds weight (g)**: Randomly 100 seed from the five plants yield were weighted and recorded as 100 seed weight.

**Grain yield (kg $ha^{-1}$)**: Grain yield of these five plants was converted to the total number of plants in the plot and then converted to hectares.

### Data analysis using software

Analysis of variance was found with R software (R i386 4.0.2) include the Duncan's multiple range test (5%) to separate significant differences in the means of the treatments.

## Result and Discussion

**Plant height (cm):** Result from the research indicated that the effects of different poultry manure

were significant (Table 1). Maximum plant height (70.10) observed at T3 treatment followed by T2 and T1 and the minimum plant height (60. 33) recorded at T0 (control) Fig 2(a). This may be due to the macro nutrients that are in the content of poultry manure which are released from the manure by decomposing for the plant growth during the growth period. This result is in harmony with (Chiezey & Odunze, 2009) who found maximum plant height with increasing of poultry manure level and (Cevheri & Yilmaz, 2018). He recorded the maximum plant height at 2000Kg ha $^{-1}$ cattle manure followed by 1500, 1000, 500 and 0 kg ha$^{-1}$.

Table 1. Mean square of ANOVA for the Growth, Yield and Yield attributes of soybean

| Source of Variance | DF | Plant height | No. Branches plant$^{-1}$ | No. Pods plant$^{-1}$ | No. Seeds plant$^{-1}$ | 100 seeds weight | Grain yield |
|---|---|---|---|---|---|---|---|
| | | MS | MS | MS | MS | MS | MS |
| Replication | 2 | 2.33 | 1.96 | 2.61 | 0.005 | 0.003 | 351.64 |
| Poultry Manure | 3 | 48.22** | 3.79** | 38.48** | 0.056** | 0.03$^{NS}$ | 13994.97** |
| Error | 6 | 0.55 | 0.09 | 0.56 | 0.002 | 0.02 | 41.03 |
| Total | 11 | 13.87 | 1.44 | 11.27 | 0.017 | 0.02 | 3903.12 |

DF: Degree of Freedom MS: Mean Square **: Significant at 1% level of probability NS: Non - Significant

**Number of branches**: Analysis of the variance showed that the effects of poultry manure were significant on soybean branches (Table 1). The more numbers of branches were recorded on T3 compared to T2, T1 and T0 and there was no– significant difference among the T2, T1 and T0 {Fig 2(b)}. The high number of branches in T3 might be due to a suitable supply of available nutrients at suitable time from poultry manure which helped in the production of branches during the growth stage. This result is in line with the finding of Yagoub et al. (2015) who observed the maximum number of branches in 2.4ton ha$^{-1}$ of chicken manure among the nitrogen fertilizer, compost and Jatropha.

**Number of pods plant$^{-1}$**: The analysis of variance indicated significant difference for the trait of pods plant$^{-1}$ (Table 1). The maximum number of pod plant$^{-1}$ (33.9) was recorded at T3 followed by T2 and T1 (30.63 and 27.23, respectively) and the minimum was recorded at T0 (control) Fig 2 (c). This is due to the higher number of branches produced by more of poultry manure (4 tons ha$^{-1}$).

**Number of seed pod$^{-1}$**: Analysis of the variance revealed significant effects of poultry manure for seed pod$^{-1}$ (Table 1). Means for the seed pod$^{-1}$ showed that the highest number of seed pod$^{-1}$ was produced by T3 (2.75) treatment which was 5%, 23% and 28% higher than T2, T1 and T0, respectively Fig 3(a).

**100 seeds weight (g)**: Table 1 indicated that there were non-significant effects of poultry manure on 100 seeds weight. Means of T1 and T2 were closely higher than T0 and T4 {Fig 3(b)}. This finding is equal with Khaim et al. (2013) who founded that 3 ton poultry manure ha$^{-1}$ produced light (12.50 g) weight of 100 seeds than 1 and 1.5 ton ha$^{-1}$ poultry manure with 75% and 50% of recommended dose of commercial fertilizers, respectively and disagreement with Chiezy and Odunze (2009) who found non-significant effects of poultry manure for 100 seed weight among the treatments.

**Grain yield (Kg ha$^{-1}$)**: Grain yield is the output of fertile branches, number of pod plant$^{-1}$, number of seeds plant$^{-1}$ and so on. The effects of different levels of poultry manure were significant on yield of soybean (Table 1). High mean of grain yield (1212.95) was observed in T3 followed by T2 and T1 (1145.16 and 1138.24, respectively) and low was recorded in T0 (1046.65) Fig 3 (c). This may be due to the releasing of supplementary nutrients by decomposing of poultry manure during the growth stages of soybean. This result is supported by Mamia et al. (2018) who noticed maximum yield (2166) in poultry manure + 75% recommended dose of fertilizer against to Vermi – Compost and Bio fertilizer with recommended dose of fertilizer and sole dose of fertilizers and also harmony with Yagoub et al (2015), the result showed that chicken manure produced maximum yield over the compost, Nitrogen and Jatropha amount of manure respectively.

**Correlation among soybean yield and yield components**

The correlation among yield and yield components of soybean crop in this study is given in Fig 5. It is seen from the figure that there are positive correlation among the yield and yield components and within yield components without 100 seeds weight with all parameters. There is positive correlation among the plant height and branches (NO)(r= 0.67*), plant height and pods plant$^{-1}$(r=0.85***), plant height and seed pod$^{-1}$(r=0.84***), plant height and total yield (r= 0.93***), branches (NO) and pod plant$^{-1}$ (r=0.83***), branches (NO) and seed pod$^{-1}$(r= 0.66*), branches (NO) and total yield(r= 0.77**), pods (NO) and seed pod$^{-1}$(r= 0.88***), pods (NO) and total grain yield(r= 0.86***) and seeds pod$^{-1}$ and total yield (r= 0.77**).

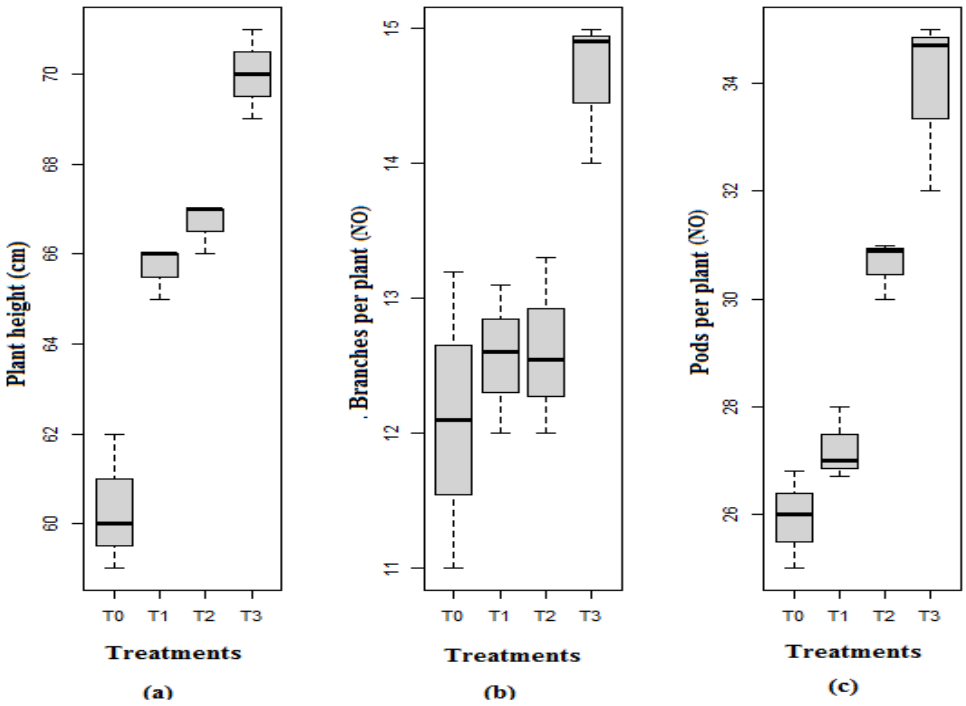


Figure 3. Response of (a) plant height (cm), (b) branches plant$^{-1}$(NO) and (c) pods plant$^{-1}$ (NO) to different level of Poultry manure.

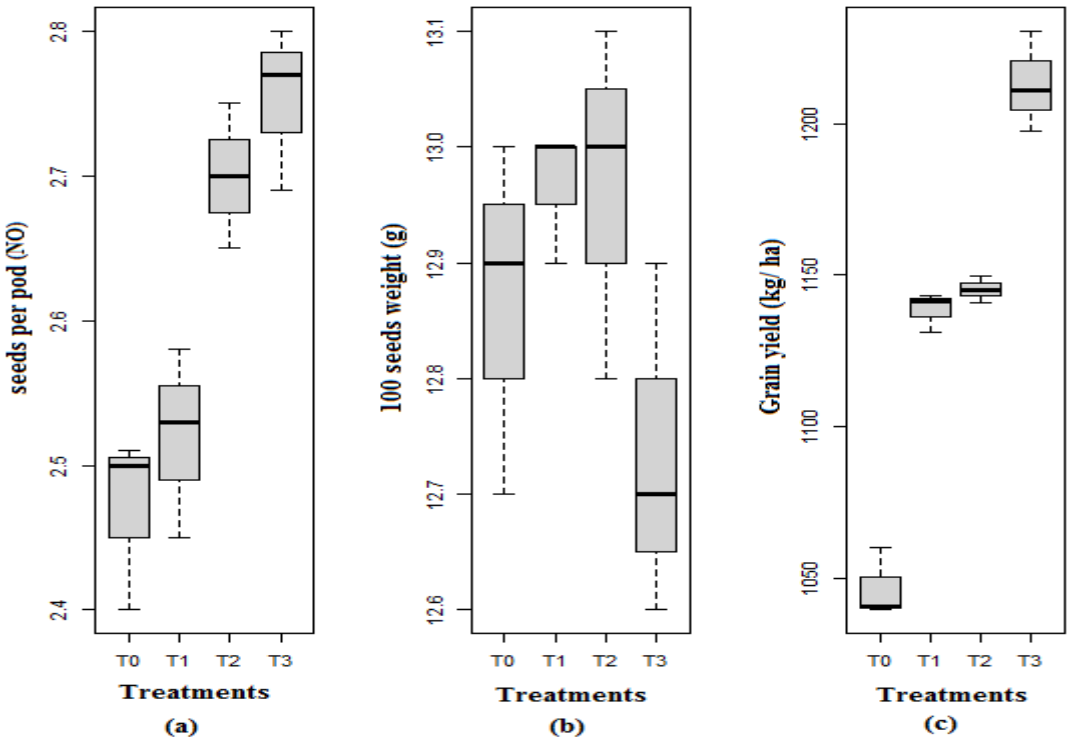


Figure 4. Response of (a) seeds pod$^{-1}$ (NO), (b) 100 seeds weight (g) and (c) total yield (kg) to different level of poultry manure.

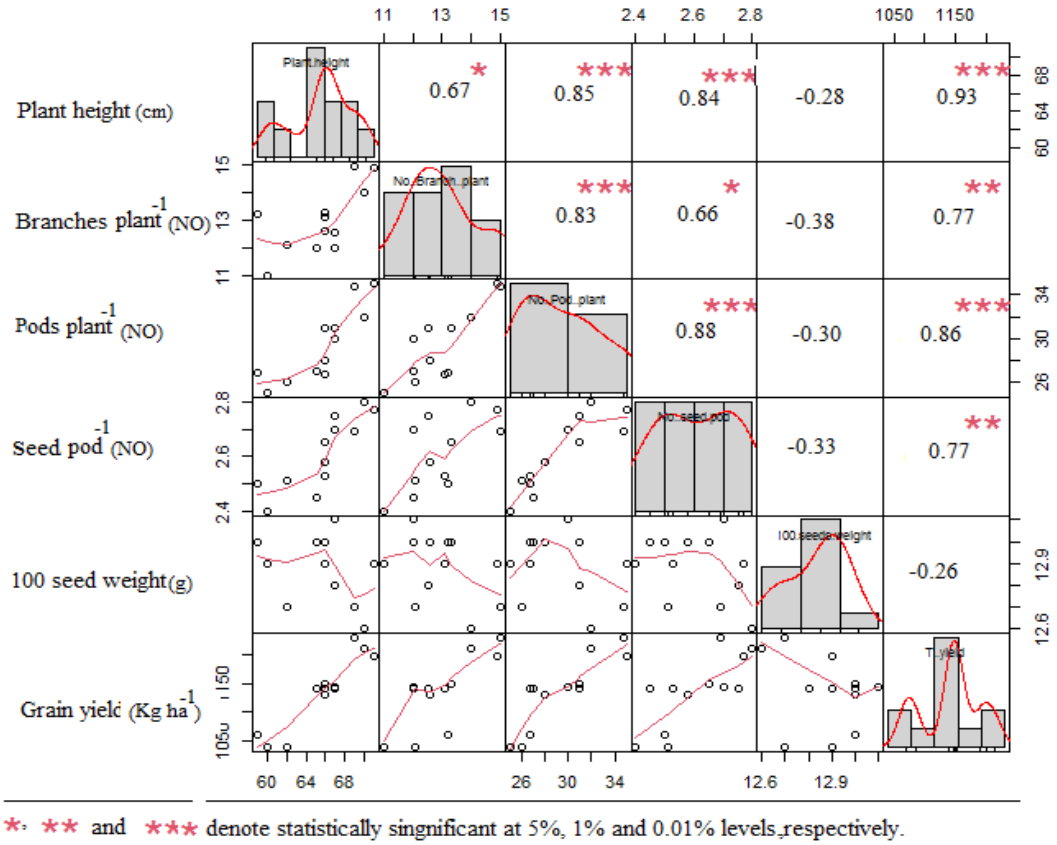


Figure 5. Correlation among yield and yield components

## Conclusion

From the current study it may be determined that the effects of different levels of poultry manure were significant. The result showed that the soybean yield and yield components were greater with increasing poultry manure. T3 (4 ton poultry manure $ha^{-1}$) produced the maximum grain yield and yield components like fertile branches, pods $plant^{-1}$ and seeds $pod^{-1}$ over the control. Likewise, soybean yield was positively correlated with yield components. So the sole application of 4 ton poultry manure $ha^{-1}$ (without any recommended dose of inorganic fertilizer) is recommended for greater yield of soybean under the Khost Agro – Ecological conditions.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Immunomodulation function of Tunceli garlic (*Allium tuncelianum*) oil in Rainbow Trout (*Oncorhynchus mykiss*)

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## Abstract

The effects of injectable Tunceli garlic oil (Allium tuncelianum) on serum response of rainbow trout were examined. Influence of intraperitoneal (i.p.) injection administration of Tunceli garlic oil (TGO) was evaluated on some immunological factors of rainbow trout. Fish weight 40 g were i.p. injected TGO at doses of 1% and 10%. Serum lysozyme activity, bactericidal activity, myeloperoxidase, protein and immunoglobulin levels were measured on days 3, 7, 14 and 21 after the TGO administration. All the immune parameters assayed remained affected when TGO was i.p. administered. Serum bactericidal activity was consistently achieved by immunizing fish with TGO. Levels of serum myeloperoxidase activity were increased in the groups of fish injected with TGO. Myeloperoxidase and bactericidal activities peaked at 14 and 21 days post-injection, respectively. Lysozyme activity was significantly increased throughout experiment in the all groups. Total protein and immunoglobulin levels increased in the 21 days post-injection although no statistically significant differences. In conclusion, these results suggest that there are functionally effects of TGO on the immune mechanisms of rainbow trout.

## Keywords

Rainbow trout, Tunceli garlic oil, Immune response, Immunity

## Introduction

Fish production is an significant and growing industry worldwide. At the same time, dense fish holding in ponds influences the health of fish. As a result, the physiological condition of productioned fish will be impacted by environmental circumstances. In this way, fish farmers have to application wary husbandry techniques. With the success of plant derivates for the control of bacterial infections, caution has focalized on plant and immunostimulants products which could have a useful impact in disease control (Ispir and Dorucu, 2005).

*Allium tuncelianum* is essentially named as *Haussk subsp. tuncelianum* Kollmann and *Allium macrochaetum* Boiss (Etoh and Simon, 2002). Even though, it is local to specially at Platos of Munzur Mountains in Ovacık district of Tunceli province Turkey, it naturally grows in the narrow region located among Erzurum and Sivas (Baktır, 2005). Owing to its resemblance to widespread garlic, it is locally called as Ovacik garlic or Tunceli garlic in the region. *A. tuncelianum* generally forms one cloved white bulb, dissimilar garlic which has a a lot cloved bulb. The flower scape of *A. tuncelianum* coils early in its prolongation, which is a original characteristic of some garlic types (Ipek et al., 2008).

Garlic and its extracts has been reported to produce various benefical effects including immunostimulator (Sahu et al., 2007), antimicrobial (Deresse, 2010; Guo et al., 2012; Ranjan et al., 2012) properties and response

against several diseases including bacteria and parasites in fish species (Colorni et al., 1998; Diab et al., 2008; Nya and Austin, 2011; Abd-El Galil and Aboelhalid, 2012; Talpur and Ikhwanuddin, 2012; Williams et al., 2012). But, to our knowledge, no previous studies TGO related to immune mechanisms of fish have been reported. This study was conducted to condition the impacts of TGO on the immune system of *Oncorhynchus mykiss*. By applicationing this kind of substrate, loss of precious fish species reasoned by pathogens in fish production might be prevented and there may also be an economic utility for fish cultivation.

## Material and Method

### Experimental fish

Rainbow trout for the experiments were obtained from a commercial fish farm in Kahramanmaras province Turkey. This fish were kept in a 225 L fibreglass tank. The fish were acclimated in experimental units for 14 days before each experiment. They were fed a commercial diet to apparent satiation once daily throughout this period. Fish with average weights of 40g were haphazardly selected and stocked at rates of 50 fish/tank for experiments at the end of the acclimation period.

### Experimental design

The Tunceli garlic (100 g) was placed in sterilized 2 liter conical flasks, separately with 1 L of corn oil and mixed good. Conical flasks were tightly covered with aluminum foil. They kept for 15 days at room temperature and agitated daily to ensure complete digestion. Then extracts were filtered through sterile muslin cloth.

Four experiments were carried out to measure immune parameters to experimental and control groups fish. Two hundred fish utilized in each experiment were divided into four groups (A, B, C and D) with 50 fish in each group. Each group was non-injected (control group A), injected with sunflower oil (control group B) or the injected with Tunceli garlic at a rate of 1 and 10% (groups C and D respectively). The analysis were performed with ten fish from each groups at 3,7, 14 and 21 days of injection. No feeding was done on sampling days. Blood was collected from the caudal. For serum separation, blood was transferred into serological tubes. The tubes were placed at room temperature for two hours, then overnight at 4ºC. The samples centrifuged for 10 min at 2500 rpm. Serum collected and stored at -20ºC.

### Immunology study

Serum antibacterial activity to *Yersinia ruckeri* was determined according to Zhang et al. (2008). According to Sahoo et al., (2005) and Quade and Roth (1997) Total Myeloperoxidase (MPO) activity in serum was measured. Lysozyme activity was determined following the method described by Zhang et al. (2008) with a slight modification. The total protein level was determined through the Biuret method (Siwicki et al., 1994; Ispir et al., 2009). According to the method previously published by Siwicki et al. (1994) The total immunoglobulin level was determined by following. The difference in protein content before and after depletion is the serum total immunoglobulin content.

### Statistical analysis

All experiments were conducted in triplicate. Mean values and standard deviations of the data of immune parameters were calculated from the experimental data obtained. Mean significance of immune parameter for experimental groups was analyzed using analysis of variance (ANOVA; Minitab Statistical Software Release). Differences between the mean values were considered significant when $p<0.05$.

## Results

There was a statistical difference between groups was at 14d when the serum bactericidal activity levels were significantly higher ($p<0.05$) in the groups of fish injected with sunflower oil and negative control (Figure 1).

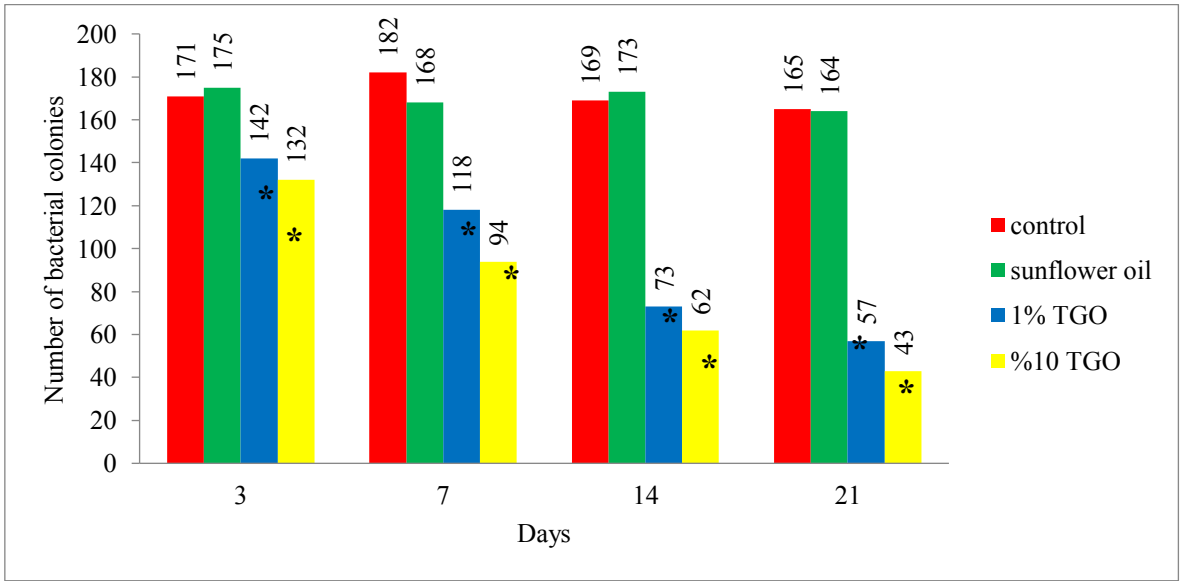


Figure 1. Serum bactericidal activity during various sampling days

The serum lysozyme activity was significantly different ($p<0.05$) between groups after intraperitoneal injection 14 d. The values obtained with serum from experimental groups fish were higher than their corresponding control at 3, 7, 14 and 21d (Figure 2).

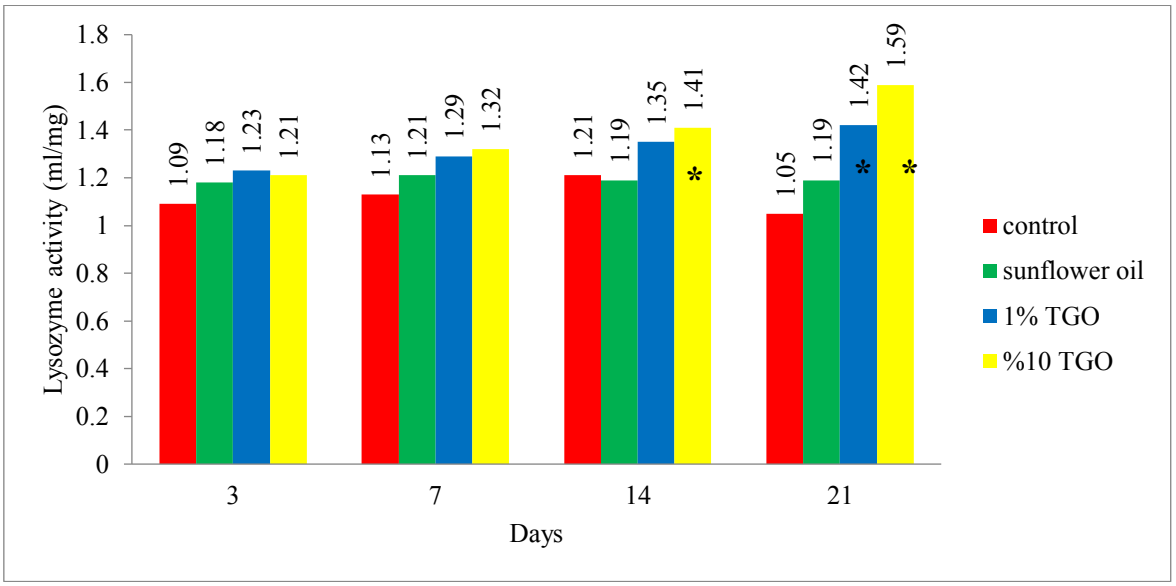


Figure 2. Lysozyme activity during various sampling days

The myeloperoxidase activity was significantly different ($p<0.05$) at 21d, with fish injected with 1.0% and 10.0% TGO having a higher activity than control fish (Figure 3). But, on days 21, MPO activity in the serum had significantly increased in comparison to control values ($p<0.05$).

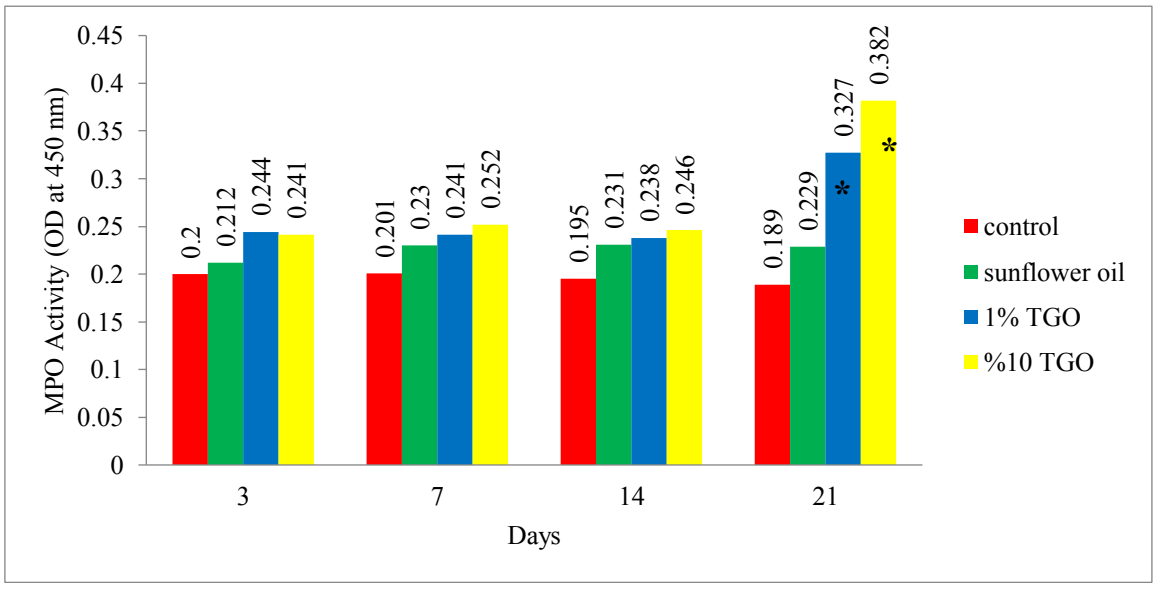


Figure 3. MPO activity during various sampling days

The total immunoglobulin and protein levels in fish injected with TGO were no statistically significant than the control group at 3, 7 and 14d. But, at 21 d post-injection for the groups injected with TGO (Figure 4 and Figure 5).

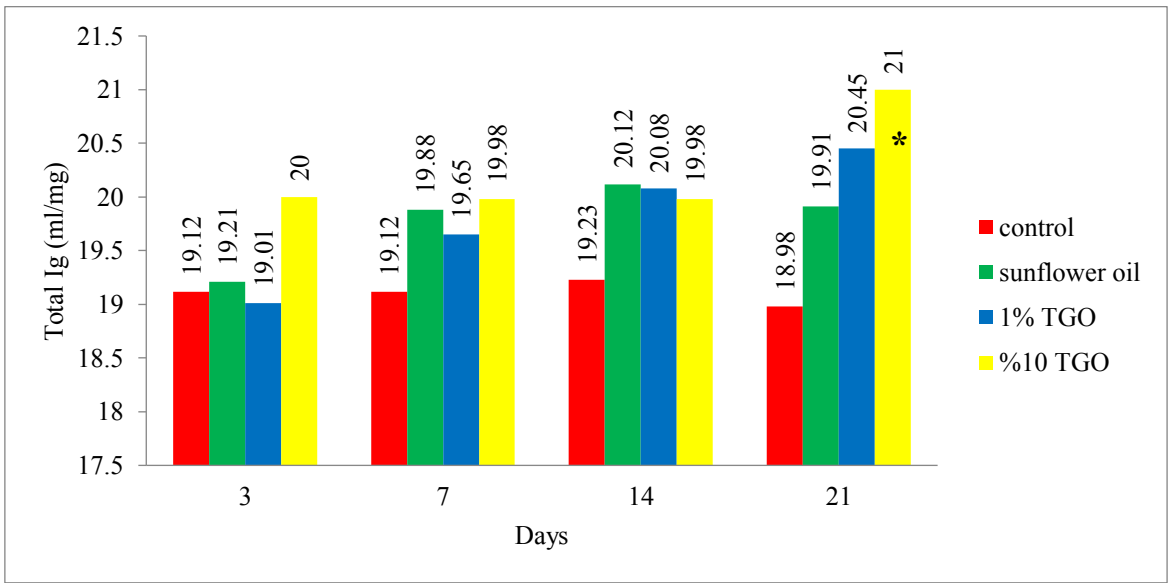


Figure 4. Total Immunoglobulin level during various sampling days

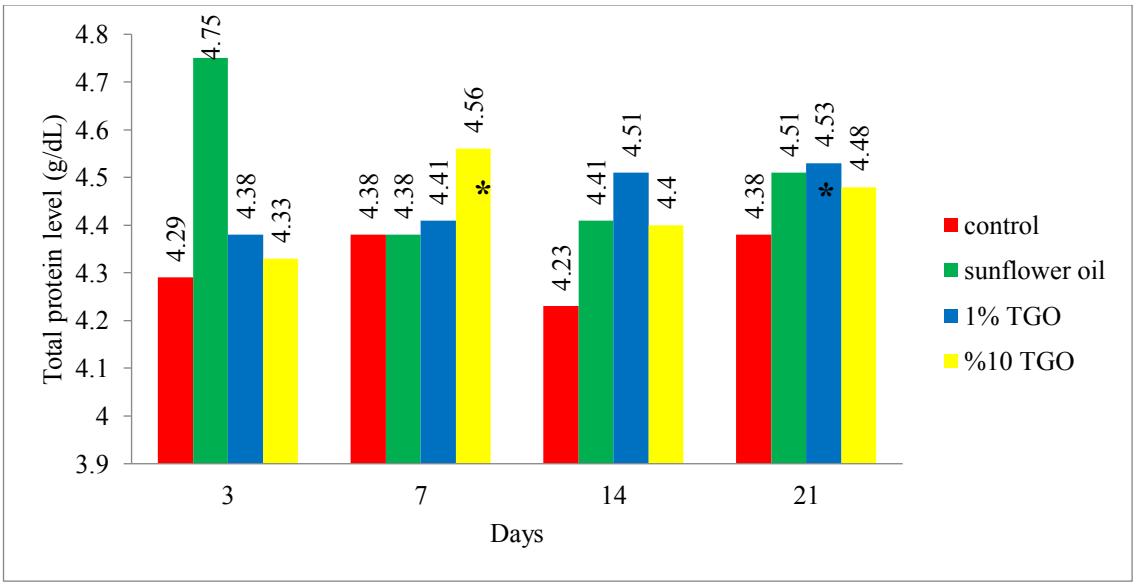


Figure 5. Total protein level during various sampling days

## Discussion

Influence of intraperitoneal injection administration of Tunceli garlic oil at doses of 1% and 10% was evaluated on some immunological factors (Bactericidal, Myeloperoxidase and Lysozyme activities; Total immunoglobulin and protein) of rainbow trout.

Rohu (*Labeo rohita*) fingerlings fed a diet additionaled with garlic for 60 days. Then this fish exposed *Aeromonas hydrophila* ($1x10^5$ CFU) by IP injection (Sahu et al., 2007). Rainbow trout (*Oncorhynchus mykiss*) fingerlings fed garlic to groups of for 14 days prior to an intraperitoneal injection challenge with *Aeromonas hydrophila* ($1x10^6$ CFU) per fish (Nya and Austin 2009). It was indicated in both of these studies that fish were fed garlic demonstrated increased serum lysozyme and bactericidal activities, and greater serum total protein. In the present study, There was a statistical difference between groups was at 14d when the serum bactericidal activity levels were significantly higher in the groups of fish injected with sunflower oil and negative control ($P<0.05$). The serum lysozyme activity was significantly different between groups after intraperitoneal injection 14 d ($P<0.05$). The values obtained with serum from experimental groups fish were higher than their corresponding control at 3, 7, 14 and 21d.

In the study by Farahi et al. (2010) was to assess the effect of *Allium sativum* on body compositions in *Oncorhynchus mykiss*. Results of Rainbow trout body compositions demonstrated that crude protein and ash increased importantly with diets containing 30g *Allium sativum.* In this study the total protein levels in fish injected with TGO were no statistically significant than the control group at 3, 7 and 14 d. But, at 21 d post-injection for the groups injected with TGO. This change is similar to the result of their work Tafalla et al. (2009), Thanikachalam et al. (2010) were found different from the changes they detected. These results agree with those obtained by Khattab et al. (2004), Shalaby et al. (2006) and Abdelhamid et al. (2002), who showed that

inclusion of Biogen in the diet increased fish protein content. In this study the total immunoglobulin in fish injected with Tunceli garlic oil were no statistically significant than the control group at 3, 7 and 14d. But, at 21 d post-injection for the groups injected with TGO. This result; Yonar, (2002), Punitha et al. (2008) and Thanikachalam et al. (2010) differed from the change they detected.

*Sophora flavescens* extract was recorded after administered diet supplemented. Tilapia an increase in myeloperoxidase immune parameters as well as resistance against *Streptococcus agalactiae* (Wu et al., 2013). In the present study, The myeloperoxidase activity was significantly different at 21d ($P<0.05$), with fish injected with 1.0% and 10.0% TGO having a higher activity than control fish. But, on days 21, MPO activity in the serum had significantly increased in comparison to control values ($P<0.05$).

## Conclusions

Plant extracts have a primarily potential utilization as an immunostimulant in fish production. As they are not act and expensive against a broad spectrum of pathogens. The preparation of plant extract is inexpensive and much easier. Many plant cultures are used as anti-viral and anti-bacterial materials. The use of plant cultures as immunostimulants in fish production systems might also be of environmental value owing to its biodegradability. In many studies, the usage of plant cultures as immunostimulant has revealed that they increase survival, growth rate and the immune responses of the fish. Owing to the beneficial impact of plant extract as immunostimulants, it can be used in fish farming as alternatives to, antibiotics, chemical drugs and vaccines.

In present study, administration of TGO enhances the some immune response parameters in rainbow trout, which can have a promising role in aquaculture to disease outbreaks and prevent diseases. Additionally, further studys on the immunostimulatory impact of TGO when administered along with feed and the preferred route of administration in the field condition for disease prevention in aquaculture are warranted.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

All animal studies were approved by KSÜZİRHADYEK and Research Institute (Protocol number: 2016/01).

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

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# Investigation of antimicrobial properties and chemical composition of different extracts of Sweet gum leaves (*Liquidambar orientalis*)

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## Abstract
Different extracts of Liquidambar orientalisleaves were tested for their antimicrobial activity against eleven bacterial and one yeast strain by disc diffusion method. The antimicrobial activity was measured by disc diffusion and MIC. After that among extracts the best antimicrobial activity was detected.Volatile components of the ethanolic extracts of leaves of sweetgum analysed by GC/MS. Ethanolic extracts of sweetgum leaves showed the best inhibition zones against Klebsiella pneumoniae (32mm). K.pneumoniae CCM 2318 showed the lowest sensitivity to 0.008 mg/mL concentration of ethanolic extracts. Ethanolic extracts of L. orientalis var. orientalis were showed the best antimicrobial activity MIC values of ethanolic extracts ranging from 0.008-64 mg.mL-1 to the tested bacteria. The GC/MS analyses allowed seven compounds to be determined; benzenepropanol (%49.30) and cinnamic acid (%35.89) were the main constituents of the of sweetgum leaves extract. All the extracts of L. orientalis leaves showed varying degrees of antimicrobial activity on the microorganisms tested. The antimicrobial activity was due to the presence the essential oils. Among the tested extracts the ethanolic extracts were the most active against the microorganisms tested compared to the reference antibiotics.

## Keywords
Antimicrobial Activity, Chemical Composition, Extract, Leaves, Sweetgum

## Introduction
Plants and derivatives have been added to different types of food to improve the flavor and organoleptic properties centuries before (Ha¨nsel and Haas., 1984). Herbal remedies used for many infectious diseases throughout the history of mankind (Rojas et al., 2003; Gottlieb et al., 2002; Narod et al., 2004).

The genus *Liquidambar* L. with Hamamelidaceae family is distributed over a wide geographical range extending from North America to East Asia. It is known that medicinal and cosmetic properties and is widely used in phytotherapy in the Mediterranean region (Sagdic et al., 2005; Ozturk et al., 2008; Beatty and Provan., 2010; Schmickl et al., 2010). There are many studies about antimicrobial activity of L. orientalis var. orientalis (Sagdic et al., 2005; Kim et al., 2008; Topal et al., 2008; Oskay et al., 2009; Yasmin et al., 2009; Sarac and Sen., 2014; Okmen et al., 2014).

The aim of this study were therefore to investigate the antimicrobial properties of six different solvent extracts from West Anatolian sweetgum and to determine the chemical compound content to find out the relationship between antimicrobial activity and the compound content. As a result of this, antimicrobial activity have been studied against some microorganisms including opportunistic pathogens. The antimicrobial activity was measured by using disk diffusion method and minimal inhibitory concentration (MIC).

## Materials and Methods
Leaves of *L. orientalis* were collected from various retail outlets in Muğla primince, West Anatolia, Turkey. The leaves were taxonomically identified at the Department of Biology, Ege University, Turkey.

Sweetgum leaves were separated, washed with distilled water, dried and then powdered finely. The leaf samples were dried on a paper in air without exposure to the sun before procurement. Thirty grams of ground air-dried leave material were shaken in 150 mL ethanol, methanol, hexane, chloroform, isopropanol or water at room temperature for 60 h in a shaker. The insoluble material was filtered by Whatman paper (No. 4) and evaporated to dryness in at 50°C. The extraction was done according to Oskay et al., 2009.

### Microbial Strains and Cultivation

Antimicrobial assays were investigated against eleven bacterial strains, five Gram-positive bacterial strains, including *S.epidermidis* ATCC 12228, *B.subtilis* ATCC 6633, *B.cereus* CCM99, *S.aureus* ATCC6538/P, *S.faecalis* ATCC8043, six Gram-negative bacterial strains, including *E.coli* ATCC 35218; *P.aeruginosa* ATCC 27853; *S.typhimurium* CCM 583; *A.hydrophila* ATCC 19570; *K.pneumoniae* CCM 2318, and yeast *C.albicans* ATCC 10239.

The bacteria strains were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 30±0.1°C, while the yeast was inoculated on yeast extract broth (Oxoid) and incubated for 48 h. Antimicrobial activity was done according to Bradshaw (1992).

### Study of Antimicrobial Effect by Disc Diffusion Method

Disc diffusion method used for antimicrobial activity which is based on the method described previously (Ali et al., 2001). Sterile paper discs (6 mm; Oxoid) were loaded with 50 µL of different amounts (0.25, 0.5 and 1 mg) of the extracts dissolved in dimethyl sulphoxide (DMSO) (Lab-Scan) and were left to dry for 12 h at 37 °C in a sterile room. Chloramphenicol (30µg) (Oxoid),erythromycin (10µg/disc), ampicillin (10 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive controls and paper discs treated with ethyl acetate, methanol and DMSO were used as a negative control. Cinnamic acid, major component of the sweetgum leaves in our study, was tested to detect the possible antimicrobial activity. For this aim, 35.89% solution of cinnamic acid (99%, Sigma-Aldrich) was prepared by dissolving in dimethyl sulfoxide (DMSO) (99.9%, Sigma- Aldrich) and used. DMSO was used as negative control to determine the sensitivity of the tested strains.

### Determination of Minimum Inhibitory Concentrations (MICs)

MICs were determined by the agar dilution method, which is based on the method described previously (Kim et al., 2005). The MICs of erythromycin (Oxoid), penicillin G and chloramphenicol (Oxoid) were also determined. A final inoculum of 1 × 104 CFU/ml was spotted onto agar plates. The plates were then incubated at 35°C for 24 h in the incubator. The MIC values of antibiotics and DMSO were tested as positive and negative control, respectively. The medium without cells was also determined to control set during MIC experiments.

### GC/MS Examination Method

The steam-distilled components were analysed by GC/MS. A HP 6890 gas chromatograph equipped with a HP-PTV and a 0.32mX0.60m HP-Innowax capillary column (0.5 µm coating) was employed for the GC analysis (Adams,1995). A computerized search was carried out using the Wiley7n.l GC/MS library and ARGEFAR GC/MS library created with authentic samples.

### Statistical Analysis

Extracts of sweetgum leaves, solvents and reference antibiotics data were taken as variables. The Shapiro-Wilk of Normal Distribution Test is applied to variable groups. The variables not having normal distribution were tested by Kruskal-Wallis, non-parametric statistical test. Mann-Whitney Tests were performed for multiple comporisons. In this way difference between zone diameters is tested.

### Results and Discussion

The disc diffusion results are presented in Table 1. Ethanolic extracts of sweetgum leaves showed the best inhibition zones against *K. pneumoniae* (32mm). The results were given in Table 1. Besides, the inhibition zone diameters of the tested extracts against the test microorganisms were shown (Table 2). Among the tested extracts, isopropanol showed the best inhibition zones against *K. pneumoniae* (23mm) in (Table 2). All of the tested extracts showed no inhibition zones against *S. typhimurium* and *S. epidermidis* in (Table 2).

Keskin and Toroglu (2011) reported that the antimicrobial activities of 12 plant species of three different extracts (ethyl acetate, acetone and methanol) were tested eight bacteria and two fungi. There are differences in the antibacterial effects of plant groups, due to differences of plant species, collection of plant season, and collection site antibacterial effects of different plants.

Basim and Basim (2013) reported that antibacterial activities of different concentration of sweet gum, *L. orientalis* var. *orientalis,*storax oil were investigated *in vitro* against 13 economically important plant pathogenic bacteria. Sagdic et al., (2005) reported that the storax was dissolved in absolute ethanol and was tested at concentrations of 10.0%, to 0.1%.. The results showed that sweet gum storax has antibacterial activity against many bacteria at different concentrations against some microorganisms at different concentrations of sweet gum storax.

Ampicilin (10µg/disc), penicillin G (10µg/disc),erythromycin(15µg/disc),chloramphnicol (30µg) and nystatin (30µg/disc) were used as positive control in Table 3. Ampicilin, penicillin and chloramphenicol very strongly inhibited the growth of *B.subtilis* whereas, erythromycin exhibited a very big zone of inhibition against *S. faecalis*. Nystatin weakly inhibited the growth of *C. albicans*.

MIC results were given in Table 4. When we compared to antibiotics with ethanolic extracts, only *K.pneumoniae* CCM 2318 showed the best antimicrobial activity (0.008 mg/mL).

Oskay et al. (2009), reported that the lowest MIC obtained with ethanolic extract *of L. orientalis* was 9.4 $mg.mL^{-1}$ for *P. Aeruginosa* and *S. pneumoniae*, whereas the highest MIC was 13,4. $mg.mL^{-1}$ for *L. orientalis* extracts against *E.coli* ATCC11229. Oskay and Sari (2007) reported that MIC value of *L.orientalis* displayed 8mg/mL against MRSA and 14.2 $mg.mL^{-1}$ against *E. coli*. Keskin and Toroglu (2011) reported that there are differences in the antibacterial effects of

microorganisms to some plants, due to the cell wall structure, species and subspecies.

Alcohols, aldehydes, fatty acid derivatives, terpenoids, and phenolics) have antifungal,antibacterial, insecticidal or nematicidal activities (Park and Shin., 2005; Park et al., 2005; Lee et al.,2008). Chemical components of *L. orientalis* resin have been well studied, and main components were styrene, α-pinene, and β-pinene. However, main components of our analysis were cinnamic acid (35.89%) and benzenepropanol (49.30%) (Fernandez et al., 2005). Cinnamic acid and benzenepropanol are well known antimicrobial compounds isolated from different plant species (Naz et al., 2006; Jananie et al., 2011; Sova, 2012).

Table 1. Antimicrobial activity of sweetgum leaves (*Liquidambar orientalis* var. *orientalis*) extracts against test microorganisms by disc diffusion method

| | Sweetgum Leaves Extract (mm) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | Methanol | | | Ethanol | | | Hexane | | | Chloroform | | | Isoprapanol | | | Water | | |
| *S.faecalis* | M | W | D | E | W | D | H | W | D | C | W | D | I | W | D | W | | D |
| | 15 | 10 | 9 | 21 | 11 | 8 | 9 | 12 | 10 | 11 | - | - | 17 | - | 8 | 8 | - | |
| *S. typhimurium* | 22 | 8 | 10 | 24 | 9 | 9 | 9 | 10 | 8 | 8 | 9 | 7 | 14 | 7 | 7 | 10 | 10 | |
| *E. coli* | 17 | 12 | 9 | 20 | 10 | 10 | - | 10 | 9 | - | - | 10 | 14 | - | - | - | 10 | |
| *P. aeroginosa* | 16 | 8 | 9 | 24 | 9 | 9 | 9 | 8 | 9 | 7 | 10 | 8 | 15 | 12 | 7 | - | 10 | |
| *A.hydrophila* | 19 | 9 | 8 | 14 | 9 | 10 | 7 | 7 | 8 | 9 | 17 | 6 | 17 | 7 | 8 | 8 | 7 | |
| *S.epidermidis* | 23 | 6 | 7 | 24 | 6 | 8 | 11 | 9 | 8 | 8 | 8 | 6 | 10 | - | 8 | - | - | |
| *S.aureus* | 17 | 9 | 8 | 19 | 9 | - | 10 | - | - | 10 | - | 10 | 13 | - | - | - | 10 | |
| *K.pneumoniae* | 19 | 11 | 9 | 32 | 10 | 11 | 9 | 10 | 8 | 9 | - | - | 23 | - | - | - | - | |
| *B.cereus* | 19 | 8 | 11 | 17 | 9 | 9 | 9 | 6 | 9 | 10 | 6 | - | 13 | 6 | 8 | - | 10 | |
| *B.subtilis* | 23 | 7 | 9 | 23 | - | 10 | - | 10 | 9 | 13 | - | 11 | 14 | 8 | 8 | - | 8 | |
| *C.albicans* | 8 | - | 7 | 15 | - | - | 7 | - | 8 | 11 | 13 | - | 18 | 10 | 7 | 8 | 14 | |

M;Me Ethanol E; Ethanol H; Hexane C; Chloroform I; Isoprapanol W; Water D; %10DMSO

Table 2. Antimicrobial activity of six different chemical against tested bacteria by disc diffusion method

| Microorganisms | M<br>mm | E<br>mm | H<br>mm | C<br>mm | I<br>mm | D<br>mm |
|---|---|---|---|---|---|---|
| *S.faecalis* | 14 | 13 | - | 9 | 11 | - |
| *S.typhimurium* | - | - | - | - | - | - |
| *E.coli* | - | - | - | 11 | 15 | - |
| *P.aeruginosa* | 10 | 10 | - | - | 13 | 9 |
| *A.hydrophyla* | 7 | 8 | - | 7 | 10 | 9 |
| *S.epidermidis* | - | - | - | - | - | - |
| *S.aureus* | 17 | 14 | - | - | 21 | 16 |
| *K.pneumoniae* | 9 | 10 | - | 8 | 23 | 10 |
| *B.cereus* | - | 9 | 8 | - | 12 | 8 |
| *B.subtilis* | 17 | 17 | 11 | - | 12 | 11 |
| *C.albicans* | 18 | 11 | - | - | 12 | 11 |

M; Methanol E; Ethanol H; Hexane C; Chloroform I; Isopropanol D; DMSO

Table 3. Inhibition zone diameters of the references antibiotics against test microorganisms

| Microorganisms | A mm | P mm | E mm | C mm | N mm |
|---|---|---|---|---|---|
| *S.faecalis* | 28 | 32 | 27 | 23 | - |
| *E.coli* | 29 | 32 | 24 | 33 | - |
| *P.aeruginosa* | 28 | 33 | 22 | 28 | - |
| *A.hydropyhila* | 27 | 31 | 25 | 26 | - |
| *S.epidermidis* | 23 | 30 | 23 | 25 | - |
| *S.aureus* | 29 | 27 | 24 | 20 | - |
| *K.pneumoniae* | 28 | 29 | 26 | 25 | - |
| *B.cereus* | 26 | 30 | 25 | 24 | - |
| *B.subtilis* | 32 | 35 | 16 | 30 | |
| *C.albicans* | - | - | - | - | 12 |

A; Ampicillin (10µg.disc$^{-1}$) P; Penicillin (30µg.disc$^{-1}$) E;(Erythromycin)
C; Chloramphenicol (30µg.disc$^{-1}$) (30µg.disc$^{-1}$) N; Nystatin (30µg.disc$^{-1}$)

Table 4. MICs of the ethanolic extracts of leaves of sweetgum,erythromycin,chloramphenicol, nystatin against test microorganisms

| Microorganisms / Antibiotics | MIC (mg.mL$^{-1}$) | | | |
|---|---|---|---|---|
| | Et | E | C | N |
| *S.faecalis ATCC 8043* | 4 | 4 | 2 | - |
| *S. typhimurium* CCM 583 | 2 | 0,016 | 4 | - |
| *E. coli* ATCC 352182 | 4 | 2 | 0,008 | - |
| *P. aeroginosa* ATCC 27853 | 2 | 4 | 0,016 | - |
| *A. hydrophila* ATCC 19570 | 64 | 2 | 0,16 | - |
| *S. epidermidis* ATCC 12228 | 2 | 4 | 0,16 | |
| *S. aureus* ATCC 6538/P | 16 | 2 | 8 | - |
| *K. pneumoniae* CCM 2318 | 0,008 | 2 | 2 | - |
| *B. cereus* CCM 99 | 16 | 2 | 4 | - |
| *B. subtilis* ATCC 6633 | 2 | 32 | 0,008 | - |
| *C. albicans* ATCC 10239 | 64 | - | - | 16 |

Et; Ethanol, E; erythromycin C; chloramphenicol N; nystatin

Table 5. Volatile components of the ethanolic extracts of leaves of sweetgum (GC-MS analysis)

| Component[a] | Area (%) | Rt[b] |
|---|---|---|
| Acetophenone | 3.4 | 21.57 |
| 1-Phenylethanol | 1.93 | 26.19 |
| Benzenepropanol | 49.30 | 32.56 |
| 1-Amino-2 Acetoamino 3 flurobenzene | 3.87 | 34.38 |
| 1,1 (Dimethyl) spiro (2,4) hepta -4- ene | 2.37 | 35.77 |
| Carvacrol | 2.96 | 36.76 |
| Cinnamic acid | 35.89 | 38.45 |
| Undefined | 0.64 | |

[a]Components listed in order of elution from a HP-1capillary column
[b] Retention time (as min)

Table 6. Antimicrobial activity of cinnamic acid against tested microorganisms by disc diffusion method

| Microorganisms | S.f | S.t | E.c | P.a | A.h | S.e | S.a | K.p | B.c | B.s | C.a |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhibition zones | 15 | 20 | 16 | 14 | 19 | 21 | 17 | 16 | 15 | 20 | 8 | 8 |

S.f *Streptococcus faecalis* ATCC 8043, S.t *Salmonella typhimurium* CCM 583, E.c *Escherichia coli* ATCC 35218, P.a *Pseudomonas aeruginosa* ATCC 27853, A.h *Aeromonas hydrophila* ATCC 19570, S.e *Staphylococcus epidermidis* ATCC 12228, S.a *Staphylococcus aureus* ATCC 6538/P, K.p *Klebsiella pneumoniae* CCM 2318, B.c *Bacillus cereus* CCM 99, B.s *Bacillus subtilis* ATCC 6633, C.a *Candida albicans*

The results of inhibition zones of cinnamic acid showed 8 to 21 mm against tested microorganisms like tested extracts in table 6. It can be suggested that antimicrobial activity of sweetgum leaves based on cinnamic acid.

## Conclusion

All the extracts of sweetgum showed varying degrees of antimicrobial activity on the microorganisms tested. To see differences between variables, pairwise comparisons were performed by applying Mann-Whitney Test. We have tested commercial cinnamic acid with our tested microorganisms. As a result, in our work, there is no statistically a significant difference between ethanolic extract and cinnamic acid (P=0.138). Therefore, it has been suggested that cinnamic acid compound of ethanolic extracts from sweetgum might be used as alternative antimicrobial natural substances and also play a great role in the discovery of new drugs.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Determination of sowing date of Crambe (*Crambe abyssinica* L.) in Northern Türkiye

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## Abstract
This research was carried out by sowing it in the winter season in 2019-2020 in order to determine the effects of sowing dates on some agricultural and technological characteristics of the crambe in Samsun ecological conditions. The research was carried out with 3 replications of divided plots in randomized blocks and crambe genotypes (NSL-74257 and PI-384530) were placed in main plots and sowing dates (October 17, November 1, November 16, December 1) were placed in subplots. Research result; the plant height was 72.28-83.62 cm, the number of seeds per plant was 668.73-842.54, the weight of 1000 seeds was 7.34-8.54 g, the seed yield per plant was 4.84-8.54 g. In addition, it was determined that the oil yield per plant was between 1.72-3.26 g and the oil ratio was 35.00-36.17%. Considering all the characters evaluated; It was determined that the first sowing date (17 October) is more advantageous than other sowing dates (1 November, 16 November and 1 December). However, since the data obtained are the results of field conditions and only a one-year study, it was found very early to establish a definite opinion on the effects of sowing dates on the characters studied. For this reason, it has been decided that it would be beneficial to continue the research for at least one more year in order to make an evaluation about the sowing date of the crambe in Samsun ecological conditions.

## Keywords
Crambe, Sowing time, Agricultural characters, Technological characters

## Introduction
Approximately 14% of the total oil and oil products production in the world are used in the oil industry. Vegetable oils are used as raw materials or supplementary products in the production of many products such as cleaning agents, soaps, skin creams, surface coaters, cosmetics, shampoo, resin, foam, ink (Mungan, 2005). Vegetable oil is produced industrially in Turkey from plants such as olive, sunflower, rapeseed, soybean, cotton, corn and hazelnuts. The oilseed production of Turkey, which was 1.1 million tons in 1990, increased 3.2 times (327%) and reached 3.6 million tons in 2020 (Anon, 2021). Despite this increase, oilseed production is not enough in Turkey today and Turkey imports crude oil and oilseeds by paying a significant amount of foreign currency each year. Therefore, it is necessary to follow a national agricultural policy that can support the production of oilseeds more, provide sufficient inputs and activate the production of alternative oil crops.

Crambe, a member of the *Brassicaceae* family, is a one-year industrial plant with high adaptability that can be grown both in winter and summer (Grombacher et al., 1993). Crambe seeds contain 35% oil and 26% protein. Crambe fatty acids composition consists of more than 56% erucic acid, 10.6-24.0% oleic acid, 6.2-14.2% linoleic acid, 7.2-15.8% linolenic acid and 2.3-9.4% eicosenoic acid (Yaniv et al., 1994). Crambe seed oil is not used for cooking due to its high content of erucic acid. However, oils rich in erucic acid are used in many industrial areas (Falasca et al., 2010). In recent years, crambe has been used in the production of industrial

products such as biodiesel, engine and machine lubricants, lubricant, bioplastic, nylon, cosmetics and paint industry for industrial purposes. Fixed oil obtained from crambe seed is used in adhesive, lubricant, synthetic rubber, motor oil, textile, perfume, detergent, pesticide industries, printer ink production and plastic industry (Erickson and Bassin, 1990). Glaser et al. (1997) reported that crambe oil is a very good hydraulic oil and cultivation of crambe in suitable areas is commercially important.

It has been reported that high performance is achieved in the cultivation of crambe in the region with an annual rainfall of less than 350 mm, and since it is a self-fertilizing plant, it can be grown in areas where rapeseed is grown without any problems (Castleman et al., 1999). Johnson et al. (1995) reported that the seed yield of crambe is higher in early sowing, decreases in seed yield in late sowing and that crambe is less sensitive to temperature and moisture stress than rapeseed. In addition, Köybaşı (2008) found that the plant height was 80.4-87.0 cm, the seed number per plant was 379.0-1885.8 and the oil content was found to be 22.50-34.62% in crambe. In another study, it was determined that thousand seed weight of crambe was 6.6 g, seed yield per plant was 2.3 g and oil content in seed was 13.4% (Arslan et al., 2015).

In addition to closing the vegetable oil deficit in Turkey, it is necessary to conduct researches to determine the production potential of the crambe plant in the ecological conditions of the country in order to evaluate the possibilities of use in different areas. Besides, it is extremely important to determine the effects of researches to be carried out in the conditions of that region in order to include the plant to be grown in a region in the cultivation system and to grow it efficiently and with high quality. Therefore, this research was carried out to determine the effects of sowing date as cultivation factor on some agricultural and technological characters of the crambe plant.

## Materials and Methods

### Plant Material and General Information about the Experimental Area

As plant material, two crambe genotypes were used which NSL-74257 (USA origin) and PI-384530 (Ethiopia origin). The soil structure of the research area is clayey, salt-free, rich in phosphorus and potassium, lime-free medium in organic matter and neutral with pH 7.0 (Table 1).

It has been showed that the average temperature in all months of the trial period is higher than the average of long years; the total amount of precipitation is lower than the values of long years in all months except April, May and June; sunny time is higher than the values of long years in all months except October and November, and there is no certain stability in terms of average relative humidity (Table 2).

The experimental area was plowed before sowing and the land was made ready for planting by pulling a rake to break up the clods after plowing. The field research was conducted with 3 replications according to the Split Blocks Design. In the research, 2 crambe genotypes (NSL-74257 and PI-384530) were placed in main plots and 4 sowing times (17 October, 1 November, 16 November, 1 December) sub plots. Each parcel has 6 rows of 5 meters in length, 40 cm between rows and 5 cm between plants in each row. During the field research period, practices such as weed control and irrigation were carried out as recommended in accordance with the crambe growing technique. Research data were taken from 10 plants randomly selected from each row before harvest. In the research; plant height, number of seeds per plant, 1000 seed weight and seed yield per plant analyzed as agricultural characters; oil content and oil yield per plant were evaluated as technological characters. Oil content analysis was performed using the Ankom XT15 Soxhlet Extraction System according to the method reported by AOCS 5-04 (Anon, 2021). Oil yield was recorded in grams by multiplying the oil rate determined as a result of the analysis for each parcel with the grain yield of that parcel. Statistical analysis of the agronomic and technological characters in the research was made using the MSTATC statistics program.

## Results and Discussion

### Agronomical Characters

As a result of the research, it was determined that the effect of sowing date was statistically significant ($p < 0.01$) on plant height (Table 3). When evaluated in terms of sowing date, the longest plant height was obtained at the 3rd and 4th sowing date (83.43 cm and 83.62 cm, respectively). On average, it was determined that the plant height increased as the sowing date was delayed (Figure 1a). In previous studies; It has been reported that the plant height in crambe is 40.00-120.00 cm (Davis, 1982), 68.00-128.00 cm (Wolf, 2000), 71.40 cm (Tansi et al., 2003) and 42.90-90.70 cm (Özyılmaz, 2019). These reported results are in line with the results obtained from this research. However, the results obtained for plant height in this research (72.20-83.62 cm) are shorter than the results which plant height is reported as 163.70 cm (Laghetti et al., 1995) and 93.07-103.90 cm (Huang et al., 2013).

In the research, it was determined that the effects of sowing dates and genotypes were insignificant on the number of seeds per plant (Table 3). It was determined that the average number of seeds per plant was 761.74. When evaluated in terms of sowing date, the maximum number of seeds per plant was obtained with 842.54 at the 1st sowing date. It was determined that, on average, the number of seeds per plant decreased after the 1st sowing date, increased again in the 3rd sowing date and decreased again after the 3rd sowing date (Figure 1b).

The number of seeds positively affects the climatic conditions, flowering status and seed formation on the plant. Proper humidity in seed formation in crambe has an extremely high effect on plant growth, and humidity affects seed formation time and seed number (Wolf, 2000). Findings reported by various researchers in previous studies that there were 5250.2 (Tansi et al., 2003) and 1003.7-2397.8 (Huang et al., 2013); although higher than the findings obtained from this research (668.73-842.54), this research's results are higher than the data reported as 379.0 (Köybaşı, 2008) and 58.0-377.0 (Özyılmaz, 2019).

As a result of the statistical analysis, it was determined that the effects of sowing dates and genotypes were insignificant on a thousand seed weight

(Table 3). In addition, it was determined that the thousand seed weight was 7.49 g on average.

When thousand seed weight is evaluated in terms of sowing date, it was obtained on the 1st sowing date with a maximum of 7.90 g of thousand seed weight. It was determined that the thousand seed weight decreased from the 1st sowing date and there was a partial increase in the 4th sowing date (Figure 1c). Thousand seed weight is greatly affected by the number of seeds per plant and seed size. In previous studies conducted by various researchers, thousand seed weight in crambe reported that 6.90 g (Vollmann and Ruckenbauer, 1993), 6.00 g (Laghetti et al., 1995), 6.84 g (Fontana et al., 1998), 5.70 g (Wang et al., 2000), 6.38 g (Lara-Fioreze et al., 2013), 6.86 g (Huang et al., 2013) and 2.60-8.50 g (Arslan et al., 2015). The results obtained in this research (7.34-8.54 g) are in line with the results reported previously.

When the data were evaluated in the light of statistical analysis, it was determined that the effects of sowing dates and genotypes were insignificant on the seed yield (Table 3). Besides, it was determined that the average seed yield was 6.22 g. When the seed yield was evaluated in terms of sowing date, maximum seed yield was obtained with 8.54 g at the 1st sowing date. It was determined that the seed yield decreased after the 1st sowing date, then it increased again in the 3rd sowing date and decreased again after the 3rd sowing date (Figure 1d). It has been previously reported by various researchers that the seed yield in crambe is 85.1-98.75 g (Tansi et al., 2003), 12.5-17.5 g (Çömlekçioğlu, 2005), 20.61-31.07 g (Köybaşı, 2008) and 33.78 g (Acar, 2015). These reported results are higher than the results from this research (4.84-8.54 g). This difference in seed yield may have resulted from different growing seasons and genotypes used in research.

### Technological Characters

As a result of the research, it was determined that the sowing date and genotype effects were insignificant on the oil content (Table 3). In addition, it was determined that the average oil content was 35.75%. When the oil content was evaluated in terms of sowing date, the highest oil content was obtained at the 2nd sowing date with 36.17%. In general, it was found that the oil content increased until the 2nd sowing date, decreased a little in the 3rd sowing date and then increased again at the 4th sowing date (Figure 2a). In the researches made by various researchers on crambe; oil content reported as 31.3% (Vollmann and Ruckenbauer, 1993), 30.6% (Bondioli et al., 1998), 32.8-37.9% (Fontana et al, 1998), 34.48% (Wang et al., 2000) and 16.17-39.02% (Özyılmaz, 2019). These reported data are in parallel with the data obtained in this research (35.00-36.17%). However, the findings of the researchers who reported the oil content as 28.78% (Lara-Fioreze et al., 2013) are higher than the findings obtained as a result of this research.

Table 1. Soil analysis results of the experimental area

| Chemical Analysis | | | | | | |
|---|---|---|---|---|---|---|
| | pH | % Limy ($CaCO_3$) | % Total Salt | Phosphorus ($P_2O_5$) kg $da^{-1}$ | Potassium ($K_2O$) kg $da^{-1}$ | % Organic matter |
| Result | 7.00 | 1.22 | 0.052 | 293.00 | 10.34 | 2.71 |
| Degree | Neutral | Non-calcareous | Salt-free | High | High | Medium |
| Physical Analysis | | | | | | |
| | | Clay (%) | | 47.45 | | Clayey |
| | | Sand (%) | | 29.40 | | Sandy |
| | | Silt (%) | | 23.15 | | Light silty |

Table 2. Some climate data of the experimental area (2019-2020 and last 55 years) (Anon, 2020)

| Months | Temperature (°C) | | Precipitation (mm) | | Relative Humidity (%) | | Sunny Time (hour) | |
|---|---|---|---|---|---|---|---|---|
| | 2019-2020 | Long-term | 2019-2020 | Long-term | 2019-2020 | Long-term | 2019-2020 | Long-term |
| October | 18.5 | 16.3 | 71.4 | 81.5 | 69.2 | 74.3 | 4.5 | 4.5 |
| November | 13.4 | 12.6 | 67.9 | 82.4 | 69.5 | 68.7 | 2.9 | 3.7 |
| December | 9.9 | 9.3 | 76.0 | 82.6 | 64.4 | 65.6 | 1.9 | 2.6 |
| January | 9.1 | 7.1 | 63.6 | 66.8 | 56.3 | 66.3 | 3.2 | 2.7 |
| February | 8.5 | 7.2 | 37.5 | 52.8 | 69.2 | 68.7 | 3.9 | 3.1 |
| March | 8.7 | 8.2 | 36.0 | 62.7 | 64.7 | 74.5 | 4.6 | 3.5 |
| April | 11.7 | 11.3 | 66.3 | 58.2 | 73.4 | 77.8 | 5.3 | 4.6 |
| May | 17.1 | 15.5 | 67.1 | 51.3 | 81.5 | 78.9 | 7.9 | 6.1 |
| June | 23.7 | 20.1 | 80.4 | 47.8 | 77.4 | 74.3 | 8.3 | 8.0 |
| Average | 13.4 | 11.9 | 62.9 | 65.1 | 69.5 | 72.1 | 4.7 | 4.3 |

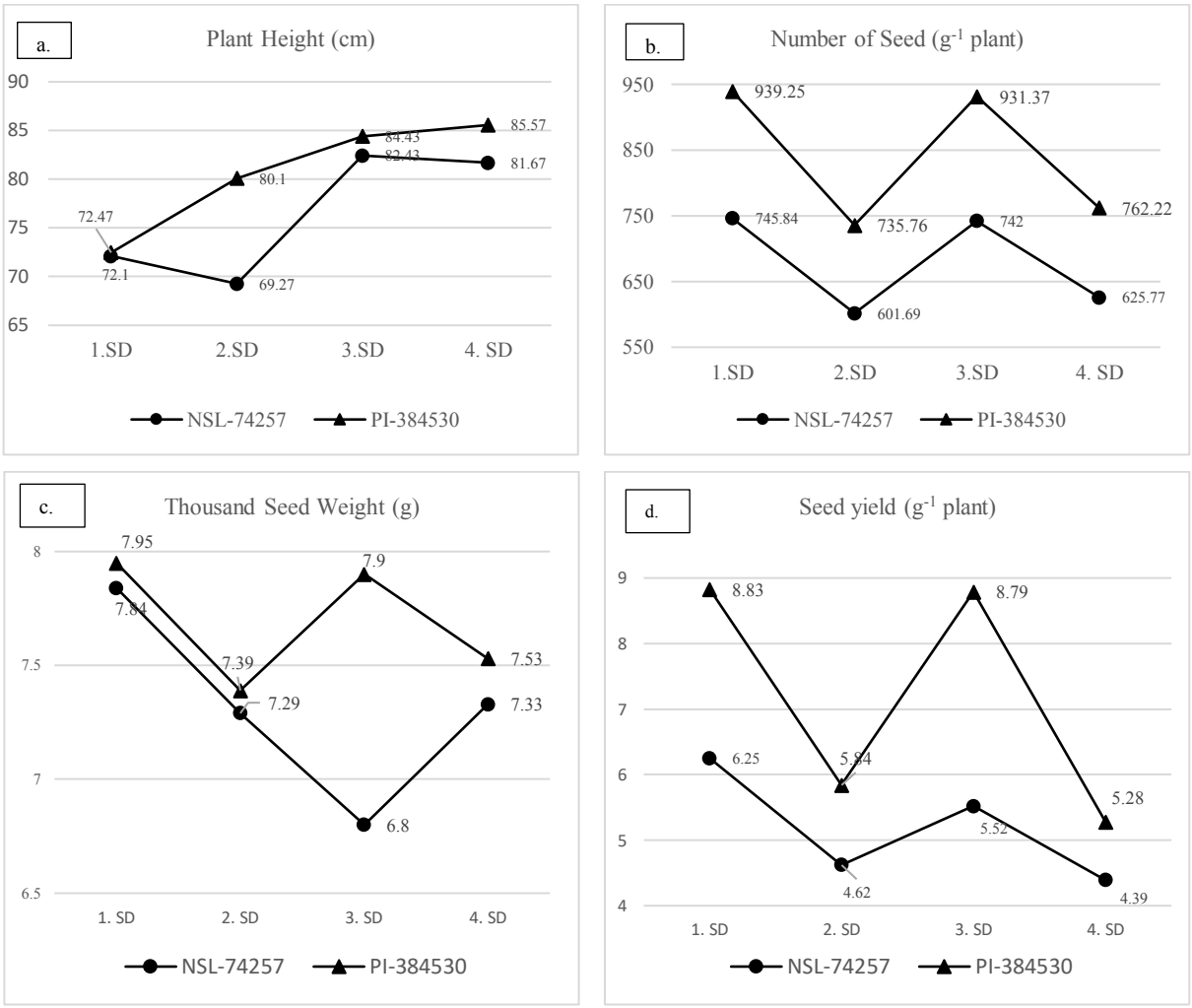


Figure 1. Change of agronomic characters of crambe genotypes at different sowing dates, a. Plant height, b. Number of seed, c. Thousand seed weight, d. Seed yield

Table 3. Variance analysis table of some agricultural and technological characters

| Sources of variation | DF | Plant Height (cm) | Number of Seeds ($g^{-1}$ plant) | Thousand Seed Weight (g) | Seed yield ($g^{-1}$ plant) | Oil Content (%) | Oil yield ($g^{-1}$ plant) |
|---|---|---|---|---|---|---|---|
| Block | 2 | 3.676 NS | 0.345 NS | 0.58 NS | 0.306 NS | 0.053 NS | 0.441 NS |
| Genotype (G) | 1 | 9.090 NS | 1.249 NS | 11.68 NS | 2.371 NS | 2.367 NS | 4.136 NS |
| Error1 | 2 | | | | | | |
| Sowing Date (SD) | 3 | 7.160** | 2.207 NS | 1.83 NS | 3.215 NS | 0.607 NS | 3.291 NS |
| G x SD | 3 | 1.098 NS | 0. 094 NS | 1.78 NS | 0.689 NS | 1.550 NS | 0.884 NS |
| Error2 | 12 | | | | | | |
| CV (%) | | 6.86 | 10.57 | 6.68 | 13.77 | 4.56 | 13.20 |

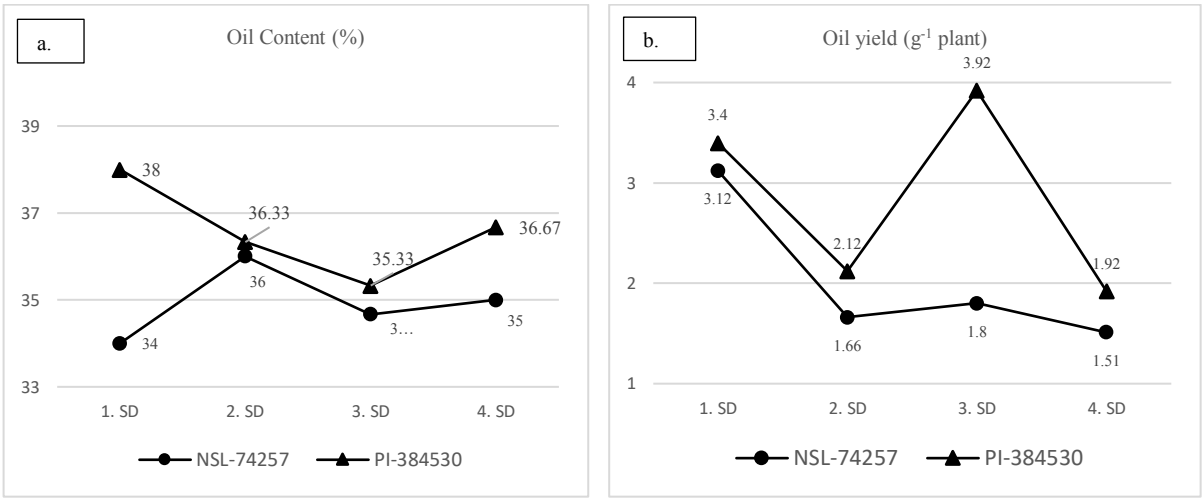


Figure 2. Change of technological characters of crambe genotypes at different sowing dates, a. Oil content, b. Oil yield

As a result of the research, it was determined that the sowing date and genotype effects were insignificant on the oil yield per plant (Table 3). In addition, it was determined that the average oil yield per plant was 2.437 g. When the oil yield per plant was evaluated in terms of sowing date, it was determined that the highest oil yield was obtained in the 1st sowing date with 3.262 g. In general, it was determined that the oil yield per plant decreased at the 1st and 2nd sowing date, increased again at the 3rd sowing date and decreased again at the 4th sowing date (Figure 2b). It was reported that the oil yield per plant varied between 0.096-1.018 g in a study conducted in crambe (Özyılmaz, 2019). In this research, it was determined that the oil yield per plant was between 1.72-3.26 g, which is higher than the value for oil yield per plant reported previously.

## Conclusion

According to the results of the research, it was determined that the plant height varied between 72.28-83.62 cm, number of seeds per plant between 668.73-842.54, thousand seed weight between 7.34-8.54 g, seed yield per plant between 4.84-8.54 g, oil content between 35.00-36.17% and oil yield per plant between 1.72-3.262 g. Considering all the characters analyzed; it was determined that the 1st sowing date (17 October) is more advantageous than other sowing dates (1 November, 16 November and 1 December). Since the research is conducted in field conditions for only one year, it is not enough to show a definite opinion about the effects of sowing date on the characters analyzed. For this reason, it was decided that it would be beneficial to continue the research for at least one more year in order to make an evaluation about the sowing date of the crambe in Samsun ecological conditions.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

O.K designed the research. K.K carried out field experiments. M.G analyzed the data. O.K and M.G wrote the manuscript. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Impact of climate on durum wheat yield (*Triticum durum* Desf.) under different cultivation and irrigation methods

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## Abstract

This study was aimed to determine the effects of different cultivation and irrigation methods on wheat in 2017-2018 and 2019-2020. The experiment design was the split-plots in randomized blocks with 3 replications. The main plots were conventional flat cultivation (CFC) and raised-bed cultivation (RBC), and sub-plots were rain-fed conditions, surface irrigation and drip irrigation. The CFC and RBC resulted in the grain yield of 5.13 and 4.33 t $ha^{-1}$, respectively. The grain yield of 5.21 and 5.55 t $ha^{-1}$ were obtained by surface irrigation and drip irrigation, respectively. The yield in CFC (16%) and drip irrigation (6%) were relatively higher than RBC and surface irrigation. Irrigation water productivity (1.72 kg $m^{-3}$) in RBC was higher compared to 1.23 kg $m^{-3}$ in CFC. The irrigation water applied was 468 and 258 mm in CFC and basin irrigation and in RBC and drip irrigation, respectively. Crop evapotranspiration was 813 and 725 mm in CFC and the basin irrigation, and in RBC under the drip irrigation, respectively. The CFC under basin irrigation was more appropriate compared to RBC and drip irrigation. Insufficient and improper distribution of rainfall and temperatures more than 30 $^{o}$C caused lower yield.

## Keywords

Cultivation systems, Durum wheat, Irrigation methods, Grain yield, Climate

## Introduction

Wheat production is restricted in arid and semi-arid regions due to insufficient rainfall or poor distribution of rainfall. Efficient use of rainfall and irrigation water is extremely important for wheat (IWMI, 2007; Cetin and Akinci 2014; FAO, 2016). The lower yields of crops such as wheat grown under the rain-fed conditions may pose a risk for increasing population and food demand. Therefore, it is inevitable to apply new production or irrigation techniques. Effective use of rainfall or supplemental irrigation have shown to overcome unstable yield levels (Tavakoli et al., 2012). In order to obtain higher grain yield, supplemental irrigation is, thus, essential. Farmers in this study area generally irrigate three or four times each season considering critical development stages of winter wheat (Cetin and Akinci, 2014). However, competition in the use of water resources between sectors is rapidly increasing. There are also considerations on the more efficient use of irrigation water in agriculture due to the higher global water use (approximately 70%) in the agricultural sector (OECD, 2020). Thus, effective use of irrigation water should be sought and implemented.

Developing planting systems that use effectively rainfall and/or irrigation water and providing moisture conservation in the soil are some of the important ways to increase water productivity (Wang et al., 2011; Zaman et al., 2017). One of these methods is the "raised-bed" cultivation system for field crops. It has been reported that it could provide moisture conservation in the soil, ease of irrigation, more water productivity and more wheat production (Sayre and Hobbs, 2004; Gursoy et al., 2010; Shao et al., 2011).

Furrow irrigated raised-bed planting consumed less water than the flat planting pattern by reducing the irrigation amount and improved control of evaporation from the top soil (Zang et al., 2007). Zaman et al. (2017) reported that the raised-bed wheat cultivation saved 14.3% water and increased grain yield 15.7% relative to the flat bed. The highest water productivity (1.81 kg $m^{-3}$) was observed in raised-bed and full irrigation condition. In another study, the ridge-furrow irrigation treatment increased significantly soil water and soil respiration in topsoil, whereas there was reduction (44.2%) in crop evapotranspiration rate and soil temperature. Therefore, the grain yield (14.6%) and water use efficiency (64.8%) significantly increased owing to the morphology of winter wheat and rooting system improvement compared to border irrigation. Ridge irrigation system combined with 75 mm deficit irrigation can be an efficient water saving strategy in semi-arid regions due to increased soil moisture across the rooting zones, a resulting in higher water productivity and wheat production (Ali et al., 2019).

The raised-bed cultivation with bed configuration of 90 cm, 4 rows and irrigation schedules (40 mm) at 0.8 times of class A pan evaporation resulted in significantly higher grain yield, straw yield, harvest index, net return, N, and K uptake (Sagar et al., 2017).

Considering different irrigation methods for wheat, drip irrigation provided 20% water saving compared to surface irrigation on wheat in Morocco. In addition, both grain yield and irrigation water productivity were higher in drip irrigation (Kharrou et al., 2011). In another study conducted in Azerbaijan, the raised-bed cultivation system saved 13% water compared to the traditional flat cultivation system.

Although there were studies on the advantages of raised-bed cultivation systems, some results showed that there was no significant differences on grain yield between raised-bed cultivation and conventional flat cultivation systems. Loper et al. (2020) reported that the planting on the flat produced 5.5 t $ha^{-1}$ (wheat); 5.6 t $ha^{-1}$ (barley) which were better than the planting on beds 4.9 t $ha^{-1}$ (wheat); 5.1 t $ha^{-1}$ (barley). In another study, irrigation amounts and nitrogen use efficiency did not show any differences in the two planting systems. Wheat yield was similar under different treatments during all the years but wheat planted on raised-beds recorded about 22.7% higher water use efficiency than under flat layout (Ram et al., 2012). Thus, some agricultural techniques needs to be developed and applied in order to use efficient irrigation water and rainfall.

This study was carried out to determine the effects of different cultivation and irrigation systems on wheat grain yield. In addition, the effects of main climatic factors such as rainfall, maximum and minimum temperatures on grain yield were also studied.

## Material and methods

### Experimental Site

This study was carried out in Southeastern Anatolia Region of Turkey, Diyarbakır (longitude: 40 ° 14' E, Latitude: 38° 01' N and elevation: 675 m). The experiments were established at Research and Experimental Station, Faculty of Agriculture, Dicle University, Diyarbakır, Turkey during the growing periods of 2017-2018 and 2019-2020. The study area has a continental climate where most of the precipitation falls in winter and there is almost no or insignificant rainfall in summer. Average yearly precipitation is 495.7 mm for long-term (1921-2021) (DMI 2020). The soils of the experimental site were clay texture (clay content of 65%) and the content of $CaCO_3$ was about 7.8%. Some chemical and physical properties of the experimental soil are given in Table 1).

### Experimental design and treatments

The experiments were carried out for 2 years in the wheat growing periods of 2017-2018 and 2019-2020. The experiment could not be conducted due to some extreme climatic conditions during the sowing period in 2018-2019, thus, the experiment in the second year was carried out in the growing season of 2019-2020.

The experiment was performed in the spit plots with three replications using randomized blocks. The main plots were conventional flat cultivation (CFC)($C_1$) and raised-bed planting (RBC) ($C_2$) and sub-plots were rain-fed conditions (RF) (Io), surface irrigation (basin irrigation for CFC, furrow irrigation for RBC) ($I_1$) and drip irrigation ($I_3$). Experimental treatments are given in Table 2. The plot size in conventional flat cultivation (treatment $C_1$) and raised-bed cultivation (treatment $C_2$) was 22.4 $m^2$ (2.8 m x 8 m).

In RBC, each furrow or ridge spacing in the plots were70 cm and 2 rows of wheat plants at 20 cm spacing on each raised-bed. Row interval was 20 cm in CFC. The beds were 40 cm wide at the top and 15 cm in height and separated by furrows 30-cm wide in RBC. Two rows of wheat were seeded on each bed at 20-cm row to row spacing (Fig. 1).

### Irrigation

Irrigation systems for surface irrigation were designed according to the characteristics of the cultivation methods. For this, basin and furrow irrigation methods were used for CFC and RBC, respectively. Irrigation was performed carried out 3 times for surface irrigations, during the stem elongation, heading and milking stages (Cetin and Akinci, 2014). The current soil moisture at the each critical stage was fullfilled to the field capacity based on soil depth of 90 cm. In the drip irrigation, irrigation started at the period of stem elongation and it was ended at the milking stage based on every 7 days and soil depth of 60 cm. In the calculation of irrigation water applied for drip irrigation, the whole area of the plots in CFC under drip irrigation was considered. However, 60% of total area of the plots in RBC under drip irrigation was considered because of space between furrows (Keller and Bleisner, 1990; Allen et al., 1998). Thus the surface of furrows which had no plants was not considered for drip irrigation.

Crop evapotranspiration was calculated using water balance equation (Allen et al., 1998).

### Agricultural applications

The experimental area was plowed deeply, the cultivator and rototiller were pulled out and the field surface was leveled and made ready for planting. In CFC plantings, wheat seeds were sown in rows of 20 cm using 200 kg $ha^{-1}$ (500 grains $m^{-2}$) in rain-fed conditions and 160 kg $ha^{-1}$ (400 grains $m^{-2}$) in the irrigated plots. In RBC, sowing was implemented using a ridge planter. Accordingly, 120 kg $ha^{-1}$ (300 grains $m^{-2}$) in the RBC and 100 kg $ha^{-1}$ (250 grains $m^{-2}$) in the irrigated plots (Kilic and Gursoy, 2010; Keskin, 2014). Sowing were implemented on 15.11.2017 and 26.11.2019 for two growing seasons. In the experiment, durum wheat variety of Sena (*Triticum durum* Desf.) developed and registered by the Faculty of Agriculture, Dicle University was used.

In the irrigated plots, 80 $P_2O_5$ kg $ha^{-1}$ for phosphorus and 150 N kg $ha^{-1}$ for nitrogen, and in the rain-fed plots, 60 $P_2O_5$ kg $ha^{-1}$ and 80 N kg $ha^{-1}$ were applied (Cetin and Akinci, 2014). Under rain-fed conditions and other irrigated plots, all of the phosphorus and half of the nitrogen were given during the sowing and the other half of the nitrogen was applied at the tillering stage of the plants. As a fertilizer source, the fertilizer of 20.20.0 (N-P-K) and urea (46% N) were used during sowing and tillering stages, respectively.

When the weeds had wide leaves reached the 2-3 leaves in the plots, some herbicides were used. Some narrow leaf weeds were collected by hand and there was no need any precaution for insects.

The harvest was implemented by a harvester machine for cereals. For evaluation, 1.0 m from the head of the plots, 1 ridge from the right-left of the plots to the ridge, and 2 rows in conventional flat cultivation were left out of the plots. The harvest was made on June 21 in 2018 and June 18 in 2020.

### Statistical analysis and evaluation

The data were analyzed using ANOVA with SPSS. The data obtained from each sampling event were analyzed separately. Duncan's multiple range test was used to determine statistically difference between the treatments at P(0.05 and 0.01) (Yurtsever, 2011). Daily climatic data for rainfall, maximum and minimum temperatures were shown in graphs and evaluated connecting yields.

Table 1. Some chemical and physical properties of soil in the experimental site

| Soil depth (cm) | pH | Org. mat. (%) | $CaCO_3$ (%) | EC (dS $m^{-1}$) | Soil texture | FC (%) | WP (%) | BD (g $cm^{-3}$) | IR (mm $h^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 0-30 | 7.7 | 1.67 | 7.8 | 0.48 | C | 35.5 | 25.5 | 1.19 | 8 |
| 30-60 | 7.9 | 1.60 | 7.8 | 0.37 | C | 35.2 | 25.3 | 1.25 | |
| 60-90 | 7.8 | -- | 8.7 | 0.20 | C | 36.4 | 27.0 | 1.27 | |

EC: Electrical conductivity, FC: Field capacity (weight (w) $w^{-1}$, %), WP: Wilting point (w $w^{-1}$, %), BD: Bulk density, IR: Infiltration rate

Table 2. Experimental treatments

| Main plots (Cultivation methods) | Sub-plots (Irrigation methods) |
|---|---|
| $C_1$: Conventional flat cultivation (CFC) | Io: Rain-fed conditions |
| $C_2$: Raised-bed cultivation (RBC) | $I_1$: Surface irrigation (border and/or furrow irrigation) |
| | $I_2$: Drip irrigation |

## Results

### Grain Yields

The experiments were planned for the growth season of 2017-2018 and 2018-2019. However, the second year of the experiment (2018-2019) could not carried out because of some extreme climatic conditions. Thus, the experiments in the second year were carried out in the growth season of 2019-2020.

The grain yields obtained depending on the experimental treatments and years are given in Table 3. According to the results of variance analysis, there was no statistically significant effect of cultivation systems on grain yield. However, the effect of different irrigation systems and rain-fed conditions on grain yield were found to be significant ($P<0.01$) and there was also significant ($P<0.05$) effects of the interaction of "irrigation systems x planting systems" on grain yield. Although the effect of cultivation systems on yield were not statistically significant on grain yield, the yield obtained from CFC was relatively higher than the yield obtained from RBC in 2018. Although the effect of irrigation systems on yield was not significant except for the rain-fed conditions, drip irrigation provided more yield compared to surface irrigation systems (border and/or furrow irrigation) (Table 4, Fig. 2). The main reason of significantly differences on the treatments in sub-plots was because of the rain-fed conditions. Accordingly, the most important factor limiting the yield was water, thus the wheat grain yield increased by means of increasing irrigation water. In addition, the effect of different irrigation systems on grain yield was not significantly different (Table 4, Fig. 2).

The results of the experiment in season of 2019-2020 were quite different compared to those in season of 2017-2018. The lowest and highest grain yield were 5.29 t $ha^{-1}$ and 7.98 t $ha^{-1}$ in this season (Table 3). As the

previous experimental results in the study, the lowest yield occurred under the rain-fed conditions and the highest yield was under the CFC and drip irrigation conditions. Accordingly, the grain yields obtained in 2019-2020 were quite higher compared with the results of the previous experimental year for all the treatments (Table 3, Fig. 2).

The effects of different cultivation and irrigation systems including rain-fed conditions on grain yield were found to be statistically significant ($P<0.01$). As the results in the season of 2017-2018, the yields in the CFC were higher than in the RBC (Fig: 2). However there was no significant difference between drip irrigation and surface irrigation applications. That is, the main difference occurred because it was rain-fed treatment. The grain yield was relatively higher in the drip irrigation compared to the surface irrigation. As the results in the season of 2017-2018, the grain yields in the season of 2019-2020 were relatively higher in the drip irrigation method compared to surface irrigation even if there was no significant difference (Table 4, Fig. 2).

According to the results of this study, there was no statistically significant differences in terms of grain yield between the RBC and CFC in 2017-2018, however the difference between yields at the cultivation methods was statistically significant in 2019-2020.

### Climatic factors, rainfall and temperature

Considering amount of rainfall during the growing season, a total of 399.7 mm of rainfall occurred during the growing period of 2017-2018 (Fig. 3a). In addition, the rainfall distribution for the months covering the crop growth period was also given in Fig. 3b. Accordingly, the rainfall during the first two months of the planting date of crop was very insignificant and very low. This was not enough to germinate and to grow for the plants. The plants have run across the winter period without appropriate development of the plants and root system in this period.

The amount and distribution of rainfall during the growing period of 2019-2020 was significantly different compared to those in the first experimental year. Accordingly, a total of 675.8 mm of rainfall occurred this year (Fig. 3a). A very high amount of rainfall occurred approximately one month after planting date, which was quite sufficient to have germination and growth of plants according to the rainfall distribution in the season of 2019-2020. For this reason, the plants has run into the winter period with a sufficient development and growth. This situation resulted in a positive effects on the development plants, thus the plants developed faster and sufficient growth in the spring period. The effect of much more amount of rainfall and irrigation resulted in the yields that is incomparably higher than those in the season of 2017-2018 (Fig. 2 and 3b). These results showed that winter wheat significantly depends on the amount and distribution of rainfall during the growing period of the plant.

According to the maximum temperatures in the growing period of the plant (Fig. 4), the maximum daily temperatures in the season of 2017-2018 were relatively higher than those in 2019-2020. This event was especially evident in the planting, emergence and development periods of the plant, and similarly, the daily maximum temperature between the 120$^{th}$ and 140$^{th}$ days after sowing were found to be quite high (Fig. 4). This condition has also explained why the yield was very low in this experimental year considering the amount and distribution of rainfall.

Considering the minimum temperatures during the growing season, the minimum temperatures between the 40$^{th}$ and 65$^{th}$ days of planting in 2017-2018 are considerably higher than those in 2019-2020. The climatic conditions might not be sufficient for the need of vernelisation in the plants, or it might has a negative effect on yield compared to the growing season of 2019-2020 (Fig. 5).

### Irrigation water and crop evapotranspiration

As given the previous part of the paper, the deficit soil moisture considering the field capacity at the date of irrigation water was applied as amount of irrigation water for all the treatments. In CFC plots in 2017-2018, irrigation water of 461.3 mm and 594.5 mm were applied in basin and drip irrigation, respectively (Table 3). In the RBC plots, irrigation water of 501.8 mm and 326.8 mm were applied in furrow irrigation and drip irrigation, respectively.

The main reason of irrigation water difference between drip irrigation in CFC and raised-bed irrigation was used the application of coefficient of 0.60 (60%) as a percentage of wetted area in RBC. It was, because, planned to irrigate the furrow ridges where the plants were located. However, there was no restriction of wetted area in the CFC plots since the plants were dense and homogenous. Thus, the wetted area was 100%. This resulted in significantly difference in irrigation water (Keller and Bliesner,1990).

In the second experimental year, in 2019-2020, irrigation water of 475.0 mm and 368.1 mm was in basin and drip irrigation in CFC, respectively. In the RBC, irrigation water of 491.8 mm and 189.1 mm were applied in furrow irrigation and drip irrigation, respectively (Table 3). The amount of rainfall and distribution during the growing period affected the amount of irrigation water applied. The amount of irrigation water applied in 2017-2018 were more than those in 2019-2020. The reason of this was occurred a rainfall of 399.7 mm and 675.8 mm in 2017-2018 and 2019-2020, respectively (Fig. 3a). That is why more irrigation water was to be applied to all the treatments in 2017-2018 and the lower yields were obtained compared to the yields in 2019-2020.

Considering the average results, the amount of irrigation water was 468.2 mm and 481.3 mm in basin and drip irrigation in CPC, respectively. The irrigation water was 496.8 and 258.0 mm in surface irrigation (furrow) and drip irrigation in the RBC, respectively.

The reason for the fact that the irrigation water applied in drip irrigation was much lower in RBC was to irrigate (wet) the ridges area where only the plants were located depending on the planting. For this reason, the wetting rate corresponded to 60% of total area and thus the amount of irrigation water was approximately 40% lower.

The values of crop evapotranspiration (ETc) for all the treatments are given in Table 3. The amounts of ETc was mainly controlled by the irrigation water applied and rainfall. The ETc in the CFC was 394.7 mm and

551.1 mm in 2017-2018 and 2019-2020, respectively. Similarly, ETc was calculated to be 392.9 mm and 527.8 mm in RBC in 2017-2018 and 2019-2020, respectively. The ETc for rain-fed conditions was almost the same with the amount of rainfall during the growing period since there was no irrigation and moisture (water) coming from the soil profile. ETc in the CFC under the basin irrigation was measured to be 729.6 and 897.0 mm in 2017-2018 and 2019-2020, respectively. In the same treatment under the drip irrigation, CFC, it has been determined as 885.2 mm and 915.3 mm in 2017-2018 and 2019-2020, respectively.

Table 4. The separate effects of the treatments on wheat grain yeild

| SP | Yield (t ha$^{-1}$) | | | WP (kg m$^{-3}$) | IWP (kg m$^{-3}$) | MP | Yield (t ha$^{-1}$) | | | WP (kg m$^{-3}$) | IWP (kg m$^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2020 | Av. | | | | 2018 | 2020 | Av. | | |
| Io | 0.86 b | 6.02 b | 3.44 | 0.74 | | $C_1$ | 2.87 | 7.39 a | 5.13 | 0.72 | 1.23 |
| $I_1$ | 3.29 a | 7.12 a | 5.21 | 0.64 | 1.08 | $C_2$ | 2.55 | 6.11 b | 4.33 | 0.67 | 1.72 |
| $I_2$ | 3.98 a | 7.12 a | 5.55 | 0.70 | 1.89 | | | | | | |

SP: Sub-plots, Av. Average, WP: Water productivity, IWP: Irrigation water productivity, MP: Main plots
$C_1$: Conventional flat cultivation, $C_2$: Raised-bed cultivation; Io: Rain-fed, $I_1$: Surface irrigation, $I_2$: Drip irrigation.
The same letter is not significant according to the Duncan's multiple range test

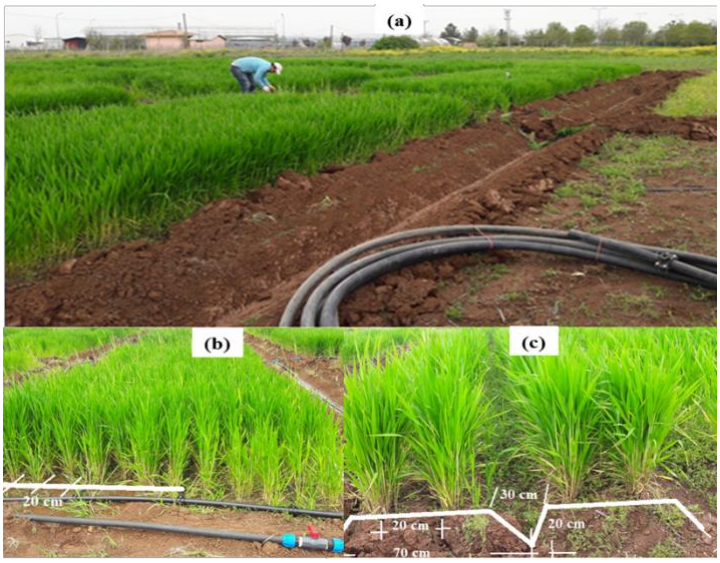


Figure 1. The general view of the experimental plots (a), conventional flat (b) and raised-bed cultivation (c)

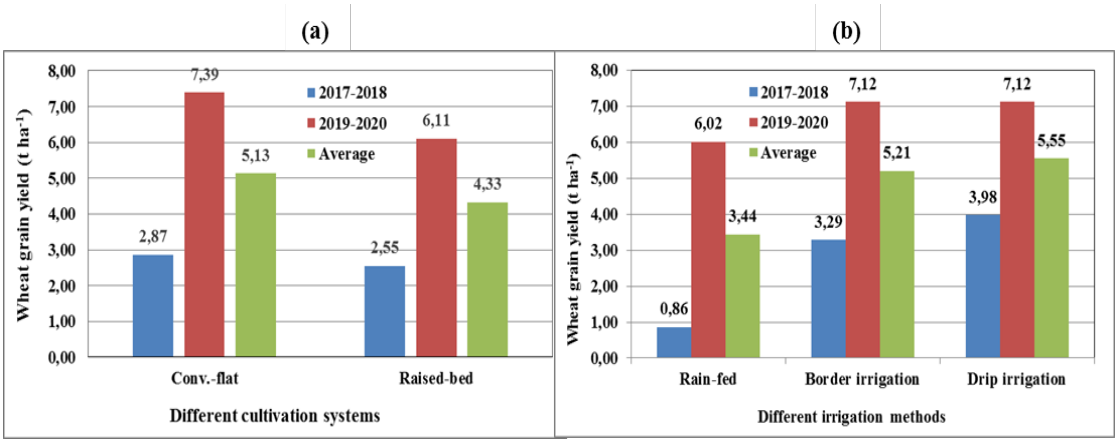


Figure 2. Effects of different cultivation (a) and different irrigation systems (b) on wheat grain yield in the experimental years.

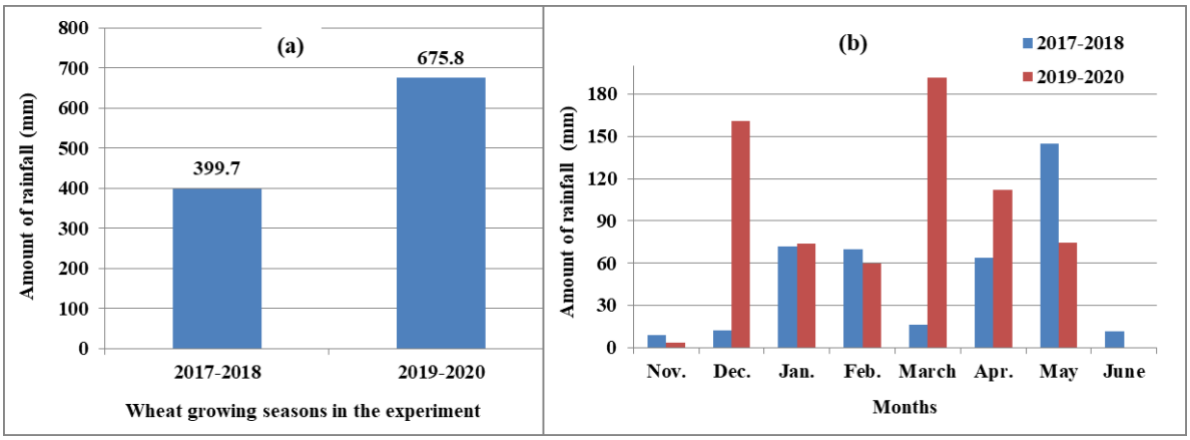


Figure 3. Total amount of rainfall during wheat growing season (a) and rainfall distribution according to the months in the experimental years

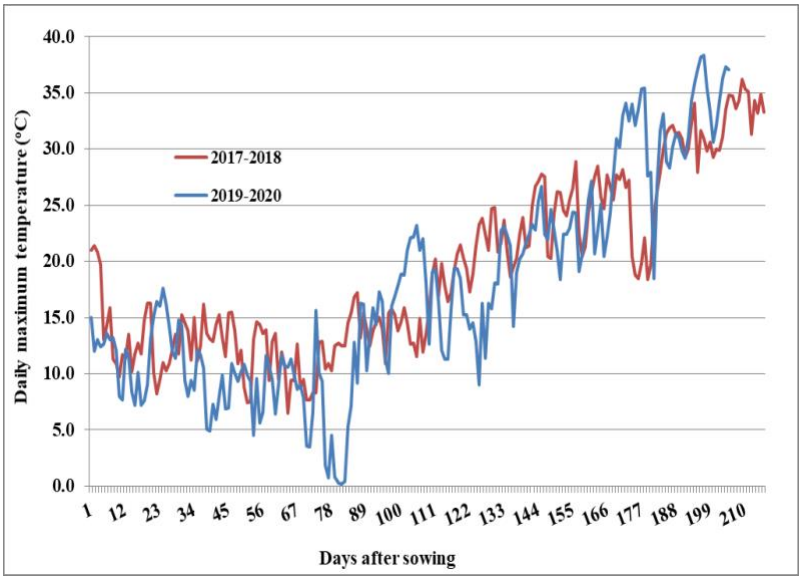


Figure 4. The variation of daily maximum temperature during the growing season in the experimental years

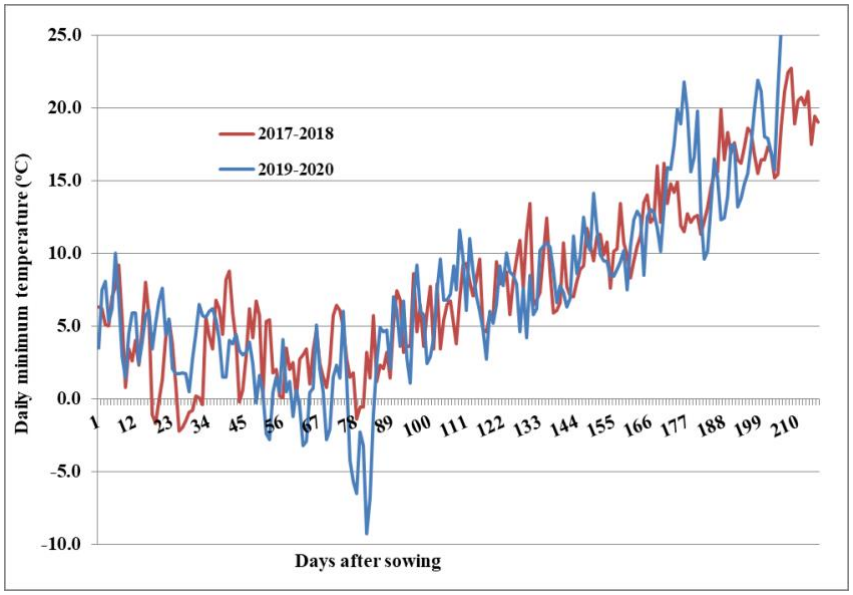


Figure 5. The variation of daily minimum temperature during the growing season in the experimental years

## Discussion

### Yields under conventional flat and raised-bed cultivation

Irrigation and/or rainfall increased wheat grain yield in all the experimental years. The most important factor limiting plant growth in arid and semi-arid climates was, because, insufficient of available water in the crop root zone (Lal, 1991; Falkenmark and Rockstrom, 1993; Cetin and Akinci, 2014). Although the irrigation implementation increased dramatically the yield, it was not sufficient to get maximum yield without rainfall and other climatic conditions in the study region (Cetin and Akinci, 2014). It is clarified that how important is the amount and distribution of rainfall during the growing period of the plant (Figure 3a and 3b). The other climatic factors such as maximum and minimum temperature and relative humidity also have a very important effect on yield.

In this study, there were no any statistically difference between CFC and RBC on grain yield even if the grain yield was relatively higher in CFC compared to RBC. One of the important reason for higher yield in CFC was used more amount of seed in CFC and this resulted in more tillering and more plant and spike number compared to those in RBC (Table 3). In addition, wide plant row spacing per unit area in ridge planting, not sowing the whole area, might cause less effective use of irrigation water due to more evaporation from the furrow surface. Thus, yields were low in the RBC. However, irrigation water productivity (1.72 kg $m^{-3}$) was higher compared to 1.23 kg $m^{-3}$ in CFC (Table 4). Although irrigation water productivity is important for water saving and efficient use of water, increasing yield is more important to get higher net return for farmers.

On the contrary to the results of this study, some successful results and advantages of the RBC have been given by the previous studies in the different regions. These advantages, were use of approximately 100 kg $ha^{-1}$ of seeds, regular use of field traffic in this system, ease and an appropriate irrigation water management, control of plant diseases in heavy soils by means of furrow irrigation (Sayre and Hobbs, 2004; Gursoy et al., 2010). Sayre (2001) reported that the RBC could provide at least 10% increase in water productivity and it could reduce production costs by 20-30% and reduce irrigation water use up to 35% under Mexican farming conditions.

In another study, permanent raised-beds demonstrated 13%, 36% and 50% higher grain yield, water saving and water productivity for the wheat crop, respectively, (Hassan et al., 2005). For equal yields beds saved 750 $m^3$ $ha^{-1}$ of water compared to flats in China for spring wheat. Requirement of the raised-bed planting system appears to be a promising way to resolve the key issues and maintain food production in Northwestern China (Zhongming et al., 2005). Wheat on raised-beds had 19.2% lower water use than on flat layout. Similarly, water use efficiency recorded in wheat on the raised-beds was 22.6% more than on the flat layout. Less water use in bed planted treatments than in flat layout was possible due to the lower amount of irrigation water (Ram et al., 2012). The permanent raised-bed plots increased wheat yields by 4.8 to 6.2% ($P < 0.05$) compared with traditional tillage (Li et al., 2014).

In another some studies, the RBC increased 12% of wheat grain yield compared to flat cultivation under farmer conditions in Pakistan. In addition, The RBC and furrow irrigation have also provided 30-35% savings in irrigation water and more benefit of 100 \$ $ha^{-1}$ (Hussain et al., 2018). Jha et al. (2017) used three irrigation methods (sprinkler, drip irrigation and flood irrigation) using different rates of field capacity (70, 60 and 50%) in wheat. According to the research results, irrigation water applied and crop evapotranspiration ranged 105-270 mm and 261-330 mm, respectively.

On the other hand, basin irrigation in the wheat had 30% more net economic return compared to furrow, sprinkler and drip irrigation because the cost of the irrigation system was lower than those irrigation systems. In addition, the economic productivity of irrigation water was higher in basin irrigation and basin irrigation for wheat irrigation was, thus, recommended (Fang et al. 2018). In a study conducted with drip irrigation in summer wheat, it was found that the highest daily water consumption was 5.18 and 7.52 mm $d^{-1}$ during the stem elongation and flowering periods, respectively. Drip irrigation increased the number of grain per spike and grain weight (Want et al., 2013).

In Pakistan, average wheat grain yield was 12% higher with ridge-furrow planting as compared to conventional planting. Wheat grain yield with ridge-furrow planting was higher than 0.1 t/ha in all districts (Hussain et al., 2018). The moisture content under the ridge irrigation was considerably improved by 29% than that of border irrigation at the flowering stage. The ridge irrigation system, moisture content significantly improved by 31% compared to border irrigation at the soil layers of 0–200 cm at three different growth stages. The ridge irrigation system increased moisture content at key growth stages, as a result significantly increased crops production (Ali et al., 2019).

CFC and RBC methods in durum wheat were tested in rain-fed and irrigated conditions under the conditions of Harran Plain of Turkey, the same region where this study was conducted. The more yield was obtained from CFC under rain-fed conditions compared to RBC and no difference was found between treatments as similar in our study. There was no also difference in yield between all cultivation methods in the irrigated conditions (Kabakci, 1999). In other studies conducted in the same region, there was no significant difference in grain yield between RBC and CFC (Gursoy et al., 2007; Kilic and Gursoy, 2010). However, the average grain yield of wheat was found to be 5615 kg $ha^{-1}$ for the farmers who planted on the RBC while it was found to be 4923 kg $ha^{-1}$ ha for the farmers who planted CFC in Mexico (Aquino, 1998). In addition, Jin et al. (2008) stated that permanent raised-bed cropping system could make a significant contribution to productivity, and they emphasized on requirements of the studies such as irrigation management, determination of suitable varieties and seed density etc.

On the other hand, the highest yield of wheat was obtained in the reduced tillage method while the lowest yield was in the CFC (Aykanat, 2009; Karaagaç et al., 2016).

One of the main reasons on less yield of wheat grain yield in the RBC compared to CFC was to be used less

seed and planting area depending on experimental design of RBC, thus this resulted in less spikes and yield per unit area (Table 2). In addition, different results obtained from this research and other previous studies might lead to the conclusion that such agronomic studies might vary depending on the region, soil and climatic conditions, therefore the results of regional research should be valid for that region.

### Crop evapotranspiration

In this study, irrigation water in drip irrigation, especially in CFC, was applied slightly more than the amount of irrigation water in surface irrigations. The main reason for this was that there were only three different periods in surface irrigation, whereas irrigation was implemented at each every 7 days in drip irrigation. Due to the application of irrigation water according to the deficit moisture in the soil and frequent irrigations in drip irrigation, the irrigation water cumulated in the soil encouraged the plant's water consumption. This increased the amount of irrigation water applied.

In some previous studies, requirement of irrigation water for wheat reported as 434 mm in Ankara-Kesikköprü (Ustun, 1990), and 430-480 mm in the Southeastern Anatolia Region where this study was carried in Turkey (Karaata, 1987; Cetin and Akinci, 2014). As a result of this study, the irrigation water requirement in the same region was almost the same and/or closer to the amounts in the previous findings.

The main reason of a high difference in ETc during the experimental years was that the amount of rainfall during the growing period was quite different. As the amount of rainfall increased, the ETc increased because deficit moisture in the soil was met almost by rainfall (Allen et al., 1998; Reynolds et al., 2000; Nagler et al., 2007), however deep infiltration increased in both irrigation water application and higher amount of rainfall in this study. The main reason of which the ETc was higher in drip irrigation was due to the higher amount of applied irrigation water, thus the plants have used water in the soil under availability conditions (Allen et al., 1998; Fries et al., 2020). The ETc of the plants was encouraged since the moisture level in the soil was higher in the drip irrigation than other surface irrigations and this resulted in higher ETc.

The difference in yield among management zones increased as crop suffered from more severe water deficit. Similar to the variation pattern of irrigation applied, the lowest seasonal ETc was obtained in the rain-fed treatment in both seasons (Li et al., 2019).

### Effects of climatic factors on grain yield

Lower rainfall caused relatively lower grain yield also under the rain-fed and all irrigation systems (drip irrigation and surface irrigations). For this reason, the yield was very low during the period of 2017-2018 under the rain-fed conditions although there was a slight increase in rainfall in the following periods (Fig. 3b), the poorly developed weak plants caused insufficient grain formation. Climatic factors such as rainfall and temperature directly affect, thus, grain yield. Climatic factors control both plant health and yield over time (Paudel et al., 2014; Ray et al., 2018).

Considering the annual amount of rainfall (495.7 mm) in the study region for the long-term (1921-2021), the season of 2019-2020 could be taken into account as an extreme year in terms of rainfall (675.8 mm) in growing season. Considering all these, wheat yield may vary from year to year according to weather conditions and agronomic practices (Yu et al., 2014). In a study, a decreasing rainfall of 28% resulted in a decrease grain yield of 27% (Hochman et al., 2017). Wheat yield and crop evapotranspiration were limited by lower rainfall during the growing season of wheat. The rainfall was more important than temperature, especially during the rapid growth period of the plant, growing up and flowering. Because, these periods were covered in which generative organs develop and grain maturity and these are sensitive to drought. Lower rainfall could produce infertility flowers and are not grown enough, thus it could resulted in lower yields (Erdelyi, 2008). Water deficit resulting from drought reduces crop yield because of its negative impacts on plant growth (Karl et al., 2009) and there is, thus, a strong relationship between rainfall and yield of wheat (Cetin and Akinci, 2014; Giunta et al., 2003; Dehgahi et al., 2014). In addition, precipitation patterns with fewer rainfall events could lead to reductions in biomass and grain yield (Oweis et al., 1998; Gooding et al., 2003) and the lowest yield was obtained in the rain-fed treatments (Li et al., 2019).

Studies have shown that water deficits applied in stem elongation and heading stages significantly decrease wheat yields (Tari, 2016), and that the drought following anthesis can negatively affect photosynthetic characteristics as well as significantly advance senescence in flag leaves (Wu et al., 2014).

The maximum temperatures occurred more than 30 $^{o}$C after flowering stage (Fig. 3) in this study. The extreme maximum temperatures affect negatively the plant physiology on pollination and grain formation. Sabello et al. (2020) showed that the plant life cycle was clearly shorter under the higher temperature conditions due to the physiological strategy of the plant to escape the high summer temperatures through early ripening of the kernels. Royo et al. (2014) reported also, the climatic zone accounted for 32.8, 28.3 and 14.5 % of variance for days to anthesis, plant height, and grain filling rate, respectively. The number of days to heading and anthesis steadily increased when moving from the warmest and driest zone of origin to the coldest and wettest one. Thus, accordingly, the increase in temperature causes a decrease in yield in hot and dry regions (Parry et al., 2004; Gregory et al., 2005; Sivakumar et al., 2005). All these findings have been verified the results in this study. High temperatures also negatively affect the assimilation and grain quality (Hatfield et al., 2011). Heat stress during the reproductive phase is, thus, more harmful than a vegetative stage due to its direct effect on grain number and dry weight (Wolenweber et al., 2003). This stage cover grain filling and heat stress at grain filling stage is one of the key factors (Luo, 2011). It is stated that average maximum temperatures greater than 30°C cause physiological stress and thus reduce the grain set or grain fill (Ferris et al., 1998; Russel et al., 2014). Thus, the increasing maximum temperature affected negatively the yield (Cetin and Akinci, 2014; Lobell et al., 2005; Luo, 2011) and high temperature decreases also mean photosynthetic rates and mean total biomass

(Monson et al., 1992). Considering the climatic conditions in this study region, higher maximum temperatures and insufficient rainfall are the main limited factors on grain yield.

The minimum temperatures between the 40th and 65th days from the sowing date in 2017-2018 were considerably higher than those in 2019-2020 (Fig.5). This temperature level might not be sufficient for the requirement of vernelisation for the plant, thus it has a negative effect on yield compared to the growing season of 2019-2020. It has been stated that the yield of wheat was directly affected by climatic parameters such as precipitation, temperature and relative humidity (Basciftci et al., 2012; Yu et al., 2014). Similarly, some researchers showed that every 1 °C temperature increase might cause a decrease of 3-66% in yield of wheat (Ozturk et al., 2017; Zhao et al., 2017). The main reason for this is the acceleration or shortening of wheat development periods with the increase in temperature (Valizadeh et al., 2014). Because, winter wheat requires a period of low temperatures (vernalization) at the beginning of crop development stage for a proper flowering time in case the wheat experiences successful grain reproduction. This requirement could make winter wheat more vulnerable to a higher temperature via insufficient vernalization (Li et al., 2013).

## Conclusions

Considering the experimental years, the amount and distribution of rainfall affected significantly on wheat grain yield under the both rain-fed and irrigated conditions. For this, while rain-fed in the dry season (399.7 mm) caused the grain yield to be as low as 1.0 t $ha^{-1}$ in 2017-2018, wet season (675.8 mm) resulted in high grain yield of up to 6.75 t $ha^{-1}$. Accordingly, rainfall during the growing period is extremely important even if all kinds of agricultural techniques are applied.

The CFC and RBC resulted in the grain yield of 5.13 and 4.33 t $ha^{-1}$ for the average data of a two year, respectively. However, irrigation water productivity (1.72 kg $m^{-3}$) was higher compared to 1.23 kg $m^{-3}$ in CFC. Similarly, the grain yield of 5.21 and 5.55 t $ha^{-1}$ were obtained surface irrigation and drip irrigation, respectively. Drip irrigation resulted in higher irrigation water productivity (1.89 kg $m^{-3}$) compared to surface irrigation (1.08 kg $m^{-3}$).

Although there were small differences between the CFC and RBC on grain yield, this was not statistically significant apart from the rain-fed conditions. Accordingly, the yield in CFC and basin irrigation was relatively higher than other treatments (RBC and drip irrigation). The irrigation water requirement was 468 and 258 mm in CFC under basin irrigation and in RBC and drip irrigation, respectively. Crop evapotranspiration was 813 and 725 mm in CFC under the basin irrigation, and in RBC under the drip irrigation, respectively. In RBC under the drip irrigation, irrigation water was applied at a lower level because the whole area was not wetted, only the ridge of the plot was wetted. In addition, one of the main reasons for the lower yield in RBC was to be used less seeds therefore there were lower plants number and spikes number per unit area.

As a result, some advantages of RBC could not be obtained in terms of water use and grain yield in this study. CFC under basin irrigation more appropriate compared to RBC and drip irrigation. Because higher grain yield is more important for farmers' returns. However, land leveling, appropriate land dimensions for basin irrigation, use of simple devices and equipments in irrigation, reducing water conveying losses will be important in order to increase water use efficiency in this cultivation.

## Compliance with Ethical Standards

### Conflict of interest

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

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Impact of seed priming on germination performance of fresh and aged seeds of Canola

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## Abstract
Priming fresh and naturally aged canola seeds with different substances and observing the seed germination properties are involved in this work. Fresh seeds and aged seeds of canola cv. "süzer" were treated with distilled water, -0.8 MPa polyethylene glycol (PEG) 6000 and 50 mM thiamine for 8 hours. Storage of canola seeds showed the loss of germination capacity compared to fresh seeds. Present findings indicate that priming can improve germination and germination speed both in fresh and aged seeds of canola. Germination percentage increased in aged seeds with distilled water priming and thiamine priming. Mean germination times were lower in the primed seeds. Seedling growth didn't improve with priming in both ages. It was concluded that germination performance improvement of aged seeds is possible with suitable priming.

## Keywords
Thiamine, PEG6000, Hydropriming, Oil seed, Storage

## Introduction
Growers and seed suppliers carry over seed lots from previous years to the following planting seasons. There is always a doubt about poor seed quality due to the storage conditions of a given seed lot. The main reasons of faster decline in seed vigour of oil seed crops are lipid autoxidation and increase in free fatty acids due to inadequate storage moisture conditions and seed bed, that induce low germination speed and seed vigour, etc ending up with poor plant stand. These two reasons cause inactivation of enzymes, denaturation of proteins and disruption of nucleic acids. Storage conditions and seed quality are very important than seed age (Elias and Copeland, 1994).

Main agricultural target for a better canola production is to get rapid and uniform germination and seedling emergence after seeds are sown. Seeds are important input cost for canola growers.

The canola germination, emergence and establishment is more sensitive compared to other oil seed crops under environmental stresses due to small seed size. Small seeds have limited food reserves causing, a short time between germination and emergence to establish a satisfactory plant stands. The producers are recommended to select seeds that have high germination speed and vigour within 3-4 months before seeding. When canola is slow or late in crop canopy formation, competing with weeds is becoming hard and reduces the herbicide performance (Mohler, 2001; Kim et al., 2011; Harker et al., 2012). High or low temperatures are the most importing constraints in winter canola cultivation especially entering the winter at rosette stage provides resistance against low temperature. For that reason high quality seed is a key factor in winter canola cultivation.

Inadequate soil moisture in seedbed is a major problem for canola seeds to establish new crops,

especially for areas having less rainfall during sowing time. Rapid germination and seedling emergence play an important part in high yield for canola cultivation.

Quality of seeds could be divergent based on storage conditions, especially if the seeds belong to different years (seed age), that effect their longevity potential (short-lived or long-lived). To increase seed invigoration of long stored or stored at unfavourable conditions hydropriming and osmopriming (hormonal priming, matri-priming and other priming) techniques are used (Windauer et al., 2007). Deterioration of seed with storage is another domain of enzyme activity which could be improved with priming. Active oxygen species like oxygen peroxide ($H_2O_2$), superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$) accumulation are related to deterioration. In order to increase seed defence system against seed deterioration related to active oxygen species and their reaction with polyunsaturated fatty acids found in cell membrane seed priming process could be helpful in many plants (Farooq et al., 2019; Khan et al., 2020).

These techniques are not only used for aforementioned stored seeds, but also newly harvested seeds. The advantages of seed priming have been revealed in both optimal and unfavourable conditions (jisha et al. 2013) for many oil seed crops such as corn, soybean and sunflower (Shrestha et al., 2019; Langeroodi and Noora, 2017; Bourioug et al., 2020).

Pre-sowing seed treatments have been used in various field crops and canola to hasten germination and improve field emergence uniformity. Polyethylene glycol (PEG), water and thiamine used successfully for many crops.

Several reports indicated uniform field emergence better crop stand as a result of improved germination and initial quality characters with different seed priming. Stored seeds have less protein content connected to oxidation of the amino acids with increased respiration. Seed deterioration due to storage conditions can be repaired with priming (Bray, 1995). Improving canola emergence significantly reduce herbicide inputs and increase seed yield.

The objective of the study was to test the impact of different priming techniques on canola seeds of different ages in terms of seed vigour tests and seedling growth parameters.

## Materials and Methods

Germination tests

One year old naturally aged and fresh seeds of a canola cultivar, Süzer, were used in this experiment. The seeds were obtained from Trakya Agricultural Research Institute. Osmotic potential of PEG-6000 was adjusted -0.8 MPa according to Michel and Kaufmann (1973) and the molecular weight of the thiamine were adjusted to 50 mM before the start of the treatment of the canola seeds.

Both fresh and aged seeds were treated with different priming solutions viz., (i) immersed in distilled water for 8 hours (hydropriming); (ii) immersed in solution of -0.8 MPa polyethylene glycol (PEG) 6000 for 8 hours, (iii) immersed in 50 mM thiamine for 8 hours. The unprimed seeds were used as control. The priming treatments were conducted at 20 °C under dark conditions for 8 hours in incubator and then treated seeds were dried at room temperature (22 ±1 °C, 45% relative humidity) for two days.

Four replicates of 50 seeds (50 x 4 = 200 seeds) were germinated between three layered rolled filter paper with 21 ml distilled water. The rolled papers with seeds were put into sealed plastic bags to avoid moisture loss. Seeds were kept in 20 ± 1 °C in dark for germination for 10 days. A seed was considered germinated when the emerging radicle was 2 mm. Germination percentage was recorded every 24 h for 10 days. Mean germination time (MGT) was calculated for the speed of germination according to ISTA (2003) rules. Shoot length, root length, seedling fresh and dry weight were measured in 10 seedlings selected randomly from each replicate after the 10th day. Dry weight was measured after drying samples in an oven at 70 °C for 48 hours.

Experimental design

The experimental design was two factors factorial (seed age × seed treatment) arranged in a completely randomized design with four replicates. The main factor was seed ages (1 year old and fresh seeds), the sub factor was seed treatments (control, hydropriming, priming with PEG 6000 and thiamine). Data for germination percentage were subjected to arcsine transformation before ANOVA was made with MSTAT-C program (Michigan State University). The differences among the means were compared with Duncan's multiple range test ($P<0.05$).

## Results and discussions

Seed is the most important part in crop life. Low quality seeds are inappropriate and lead to decrease in efficiency of other inputs. Seed aging is one of the reasons of leading low quality and seed vigour naturally declines during storage. In this study it is clear that priming can increase seed quality.

Results clearly showed that the mean germination time was 1.34 days in fresh seeds and 1.92 days in the one-year-old seeds (Table 1). Germination percentage was higher in fresh seeds (99.38 %) compared to one-year-old seeds (82.63%)

This study revealed that hydropriming and thiamine priming caused partial increases in the germination percentage of aged seeds of canola compared to control. Germination percentage was higher in fresh seeds compared to the one year old seeds. Hydropriming and priming with thiamine showed 93.75% and 94.25% germination respectively (Table 1).

A two-way interaction was discovered for MGT, GP and seedling fresh weight (Table 1). Among seed treatments hydropriming shortened the mean germination time in both seed age compared to control. In both seed ages, hydropriming and treatment with thiamine showed high germination percentage (Table 2). The hydroprimed seeds had shorter time for seed germination, due to faster water absorption and earlier beginning of metabolism processes (Kaya et al., 2006; Sağlam et al., 2010). One year old seeds had the highest root length, seedling fresh weight and seedling dry weight.

Primed seeds of both age with thiamine showed improvement compared to non-primed seeds. Especially one-year old canola seeds primed with thiamine had shorter mean germination time and higher germination percentage compared to other treatment, but not other

growth parameters were affected by the thiamine priming (Table 2). It could be attributed to thiamine priming of canola seeds that increased activity of thiamine dependent physiological reactions in germination (Neumann et al., 1999). Thiamine molecule is an incipient thiol and high uptake of exogenous thiamine into canola seeds might also stimulate the thiol metabolism like antioxidative characteristics of thiol compounds to reduce oxidative stress (Neumann et al., 1999).

Priming with distilled water and thiamine increased germination percentage of aged seeds, but without any significant difference in priming treatments of fresh seeds (Table 2).

Seedlings were shorter in PEG priming and thiamine priming compared to control and hydropriming. Also hydropriming produced more taller seedlings. Roots were longer in PEG priming.

Aged seeds price can be lower than fresh seeds and farmers can use aged seeds by increasing seed amount for sowing. Also improved priming technology to increase germination percentage of the aged seeds is needed. Germination performance improvement of aged seeds with priming treatments has been demonstrated as an effect of repair mechanisms happening during priming. The seeds in ageing process deteriorate and need repair during imbibition in order to germinate (Nascimento and Aragão, 2004). It therefore seems that aged seeds became more vigorous with appropriate priming treatments.

Table 1. Germination parameters of canola cv. Süzer at two different seed aging levels and priming treatments

| Factor | Treatment | Mean Germination Time | Germination Percentage | Seedling Length | Root Length | Seedling Fresh Weight | Seedling Dry Weight |
|---|---|---|---|---|---|---|---|
| | | days | % | cm | cm | mg plant$^{-1}$ | mg plant$^{-1}$ |
| Seed Age | Fresh (F) | 1.34 b | 99.38 a | 6.69 | 7.41 b | 51.38 b | 2.00 b |
| | One year (O) | 1.92 a | 82.63 b | 6.75 | 5.03 a | 62.44 a | 3.25 a |
| Priming | Non-Primed (N) | 1.83 a | 89.75 b | 6.79 ab | 6.18 ab | 60.63 a | 2.88 |
| | Distilled-Water (D) | 1.46 c | 93.75 a | 7.28 a | 5.53 b | 57.13 ab | 2.63 |
| | PEG 6000 (P) | 1.73 a | 86.25 b | 6.41 b | 7.28 a | 56.63 ab | 2.63 |
| | Thiamine (T) | 1.43 b | 94.25 a | 6.40 b | 5.89 b | 53.25 b | 2.38 |
| Summary of ANOVA | | | | | | | |
| Seed Age (A) | | ** | ** | NS | ** | ** | ** |
| Priming (P) | | ** | ** | * | ** | * | NS |
| A x P | | * | ** | NS | NS | ** | NS |

*$^{**}p<0.01$,* $^{**}p<0.05$, ns, not significant

Table 2. Germination parameters of fresh and one year old canola cultivar “süzer” exposed to different priming treatments

| Seed Age | Priming | Mean Germination Time | Germination Percentage | Seedling Length | Root Length | Seedling Fresh Weight | Seedling Dry Weight |
|---|---|---|---|---|---|---|---|
| | | days | % | cm | cm | mg plant$^{-1}$ | mg plant$^{-1}$ |
| Fresh | Non-Primed | 1.63 bc* | 99.00 a** | 6.71 | 5.08 | 54.50 cd** | 2.25 |
| | Distilled Water | 1.04 d | 100.00 a | 7.33 | 3.92 | 57.00 bc | 2.00 |
| | PEG 6000 | 1.57 c | 99.50 a | 6.23 | 6.36 | 47.75 d | 2.00 |
| | Thiamine | 1.14 d | 99.00 a | 6.49 | 4.78 | 46.25 d | 1.75 |
| One Year | Non-Primed | 2.03 a | 80.50 c | 6.88 | 7.28 | 66.75 a | 3.5 |
| | Distilled Water | 1.87 ab | 87.50 b | 7.22 | 7.15 | 57.25 bc | 3.25 |
| | PEG 6000 | 1.89 ab | 73.00 d | 6.59 | 8.21 | 65.50 ab | 3.25 |
| | Thiamine | 1.72 bc | 89.50 b | 6.32 | 7.00 | 60.25 abc | 3.00 |

*$^{**}p<0.01$,* $^{**}p<0.05$

## Conclusions

Hydropriming with thiamine and hydropriming results are very encouraging and showed improved germination of aged seeds. Winter canola cultivation is hard due to high or low temperature stresses that have an uneven effect on seed germination. Slow germination delays emerging and this also delays rosette stages which is an important phase for winter hardiness. Increasing seed germination and slow seed germination speed is very important point in winter canola cultivation. In order to increase seed vigour in aged or low vigour seeds of canola to have better stand in field, priming with suitable methods should be improved.

## Compliance with Ethical Standards

### Conflict of interest

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

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Overview of the lands of Dalaman agricultural enterprises using geographic information systems

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## Abstract
Dalaman Agricultural Enterprise, located in the southeast of Muğla province, was established in 1935 as a subordinate of the State Agricultural Enterprises Institution. Field agriculture has an important place in the enterprise, which has an area of approximately 33630 decares. In addition, there are citrus plants in irrigated farming areas in the enterprise. The most important data source for the operating lands is the report "Study and Mapping of Dalaman State Production Farm Soils". Environmental data and soils of the study area are handled in the report. The maps of the enterprise lands were digitized in the GIS, the soil series and important phases were determined and various thematic maps were produced. Maps created and the soil properties are a summary of the Dalaman Plain soils. The results obtained will be a base map for the studies to be carried out in the region.

## Keywords
Detailed soil survey, Geographic information system, Dalaman agricultural enterprises

## Introduction
It is necessary to systematically examine the soils, which are specified as the most important source for agricultural production in nature. In this context, fieldworks, laboratory and office studies should be handled as whole. Soil survey and mapping studies which are explained as defining the characteristics of soils in an area, classifying soils according to a standard classification system, showing the boundaries of soil types and make a prediction about the behavior of soils, determination of important physical and chemical properties of soils are the whole of evaluation and mapping studies of lands (Dinç and Senol, 2009). The reports performed for the areas where detailed soil survey and mapping are carried out, are also consider as important materials for researchers working on different disciplines.

On the national scale, many countries that have completed detailed soil maps have been revising their soil maps. However, there is no attempt at the level of country for detailed soil survey and mapping studies. On the other hand, universities, public institutions and foundations performed detailed soil survey of some regions, especially their areas of responsibility. Among the universities, University of Çukurova has carried out and has been continue to do important studies on detailed soil surveys. In this context, the studies of the lands of the Çukurova Region, the Southeastern Anatolian Region Plains, the Turkish Republic of Northern Cyprus and the lands of the agricultural enterprises affiliated to the General Directorate of Agricultural Enterprises (TİGEM) have been completed. Some of these enterprise areas are also recently updated via GIS (Dingil et. al., 2013).

In this study, Dalaman Plain, which is one of 29 different TİGEM agricultural lands located in different locations of Anatolia in Turkey, is considered. However, maps created using the technological conditions of the relevant period were only hard print. Although there are studies on different subjects belonging to the enterprise, studies on its lands have been limited (Ata and Tekin, 2001; Kaya and Kaya, 2003; Koç et.al., 2004; Dedeoğlu et.al., 2019). In this study, it is among the objectives of this study to create the database of the enterprise lands, for which detailed soil surveys have been completed before (Anonymous, 1982), to produce various thematic maps and to present them to users in other disciplines.

## Materials and Methods

### Material

Dalaman Plain, which is located within the borders of Muğla province, is bordered by Fethiye in the east, Marmaris in the west, the Mediterranean in the south and Köyceğiz in the north. Dalaman Agricultural Enterprise is located in the south of this plain adjacent to the sea (Fig 1.). When detailed soil surveys were carried out, Dalaman TİGEM was surrounded by Tersakan and Taşlı streams in the east, Dalaman Stream in the west, the Mediterranean in the south and Dalaman County Prison in the north. It is reported that the Dalaman Plain belonging to TİGEM was 43050 decares (da) of land. However, current data report that the boundaries of the TİGEM land have changed over time and are currently 33630 decares (Anonymous, 2021a). Approximately 4800 acres of land is used as Dalaman Airport, which was opened to international air traffic in 1981.

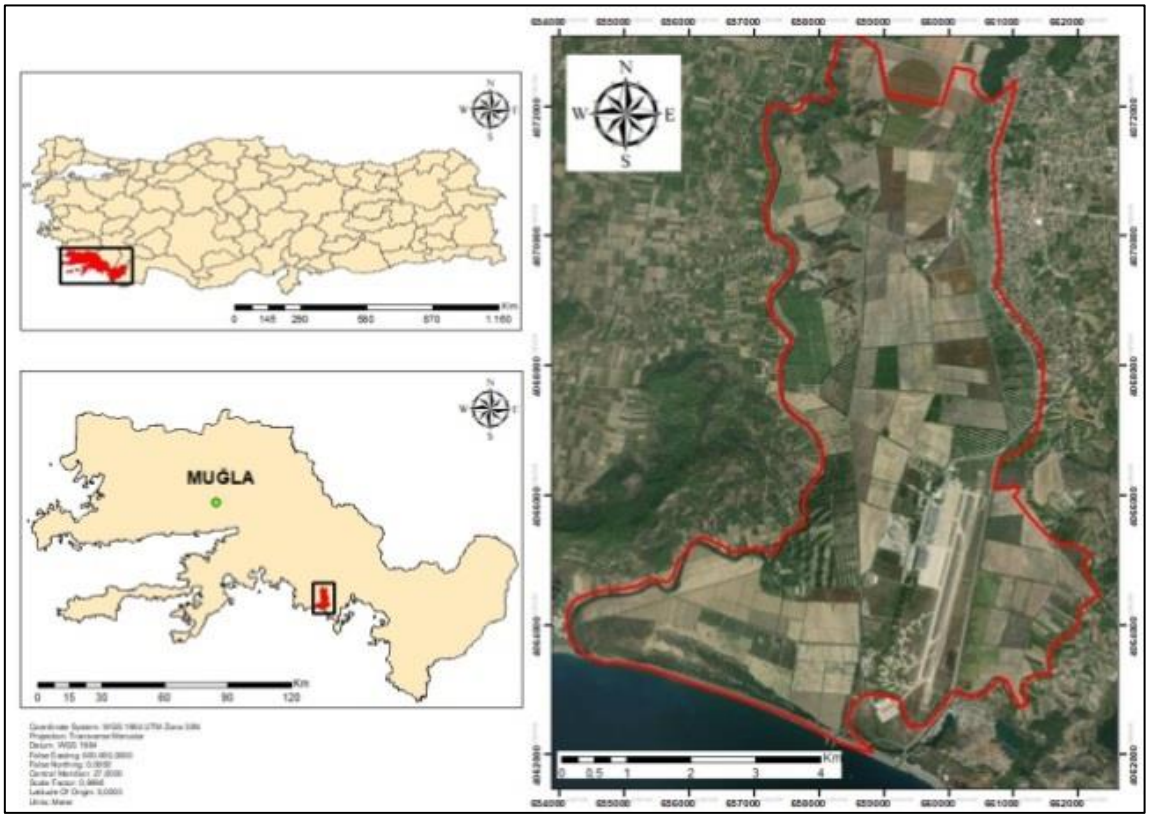


Figure 1. Geographic location of the study area

The meteorological climate of the study area is hot in summers; winters are considered mild (Anonymous, 2021b). Considering annual climatic data, the lowest precipitation is in July (11.7 mm); the highest precipitation is seen in December (265.5 mm). Average temperature values are the lowest in January (5.3°C); shows that the highest temperature is in July (26.4°C). Generally, a significant part of the plain has alluvial deposits (Keskin, 2012). The most important geomorphological formation is the alluvial fan formed as a result of the activity of the Dalaman Stream in Holocene and Pleistocene. In addition to, there are also elevations consisting of ophiolites that cover a very small area. Croplands have an important place in land use. For the purpose of certified seed production; for the production of wheat, maize and vetch, alternation crops; grain corn and oil sunflower and roughage production for livestock needs. In addition, there are citrus plants in irrigated farming areas in the enterprise. In addition, as mentioned before, Dalaman Airport also occupies an important area within the enterprise (Fig 2). There are 7 series in total in the enterprise. These series are Dalaman, Eskiköy, Adaköy, Havaalanı, Camikırı, Gökdağ and Söğüt series. All of these series are defined in Entisol order, and all series except Gökdağ series are classified in Fluvent order. Gökdağ series, on the other hand, is located in the Orthent sub-order. Some general characteristics of the series will be given together with the maps obtained in the research findings section of the study.

### Method

In this study, which was carried out in the office, the maps of the TİGEM land, for which detailed soil surveys were carried out before, were transferred to the Geographic Information Systems (GIS) and various thematic maps were produced. For this purpose, the

basic soil map, which is in the form of a printed map, was scanned on the scanner and then geographically corrected with the help of Google Earth in the GIS. Land boundaries and other mapping units were manually digitized, a database (attribute) was created and maps were produced. At this stage, ArcGIS 10.4 software was used.

## Results and Discussion

### Soil Series

7 soil series, namely Dalaman, Eskiköy, Adaköy, Havaalanı, Camikırı, Gökdağ and Söğüt series, have been defined in the TİGEM field. The distribution map of these series within the TİGEM lands is given in Figure 2. According to the inquiries made under GIS, the most widespread series soils in the study area are Dalaman and Havaalanı series. The least widespread series is the Camikırı series. The area covered by the series defined in the enterprise is given in Table 1. The Dalaman series consists of materials brought and deposited in the alluvial terraces of the old Dalaman Stream with subsequent floods. For this reason, the texture, which is partially light in the upper horizons, becomes heavier towards the depths and because they are terrace soils, there are sand and gravel deposits after 150 cm. Color is brown in the whole profile and free $CaCO_3$ is dispersed in the whole profile. Eskiköy series, which is the second series showing the most area, was formed on the alluvial terraces of Dalaman Stream, just like Dalaman series. $CaCO_3$ is high in the profile. The soils of this series also have a clayey loam texture on the surface, but have a coarser sandy clay loam texture in the lower horizons. Havalaanı series, which has the highest spread, is also among the lands affected by Dalaman Stream. The soils of the series located on the terraces of Dalaman Stream are similar to the Dalaman series, but are distinguished from the soils of this series by the presence of silty clay loam bands exceeding 30cm in thickness under the surface.

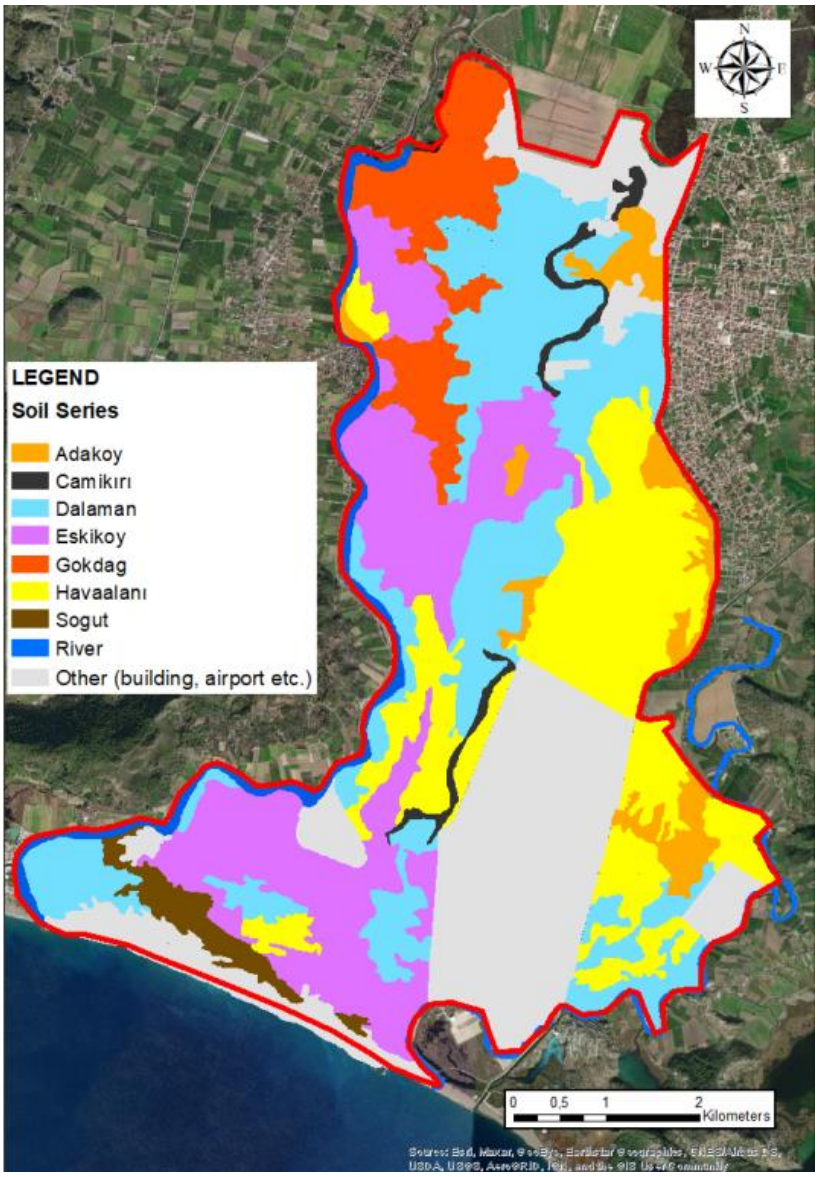


Figure 2. Distribution map of the soil series

Table 1. Defined series and total areas of the study area

| Series Name | Series Area (da) | Series Area (%) |
|---|---|---|
| Adaköy | 1826 | 4 |
| Camikırı | 629 | 1 |
| Dalaman | 9174 | 21 |
| Eskiköy | 8118 | 19 |
| Gökdağ | 2973 | 7 |
| Havaalanı | 8950 | 21 |
| Söğüt | 792 | 2 |
| Others | 10627 | 25 |
| Total Area | 43088 | 100 |

## Physiographic Units

The defined 7 soil series are located in different physiographic units. These units are the old coastal dunes, the old riverbed, the ridge, the river terrace and the uplands. Dalaman and Eskiköy series are located in the most widespread river ridge physiographic unit. Adaköy and Havaalanı series, which are among the river terrace physiographic units, are distributed in 25% of the study area. Gökdağ series high lands, Camikırı series old riverbed and Söğüt series old coastal dunes are included in physiographic units (Table 2). Physiographic unit map created in GIS is given in Figure 3.

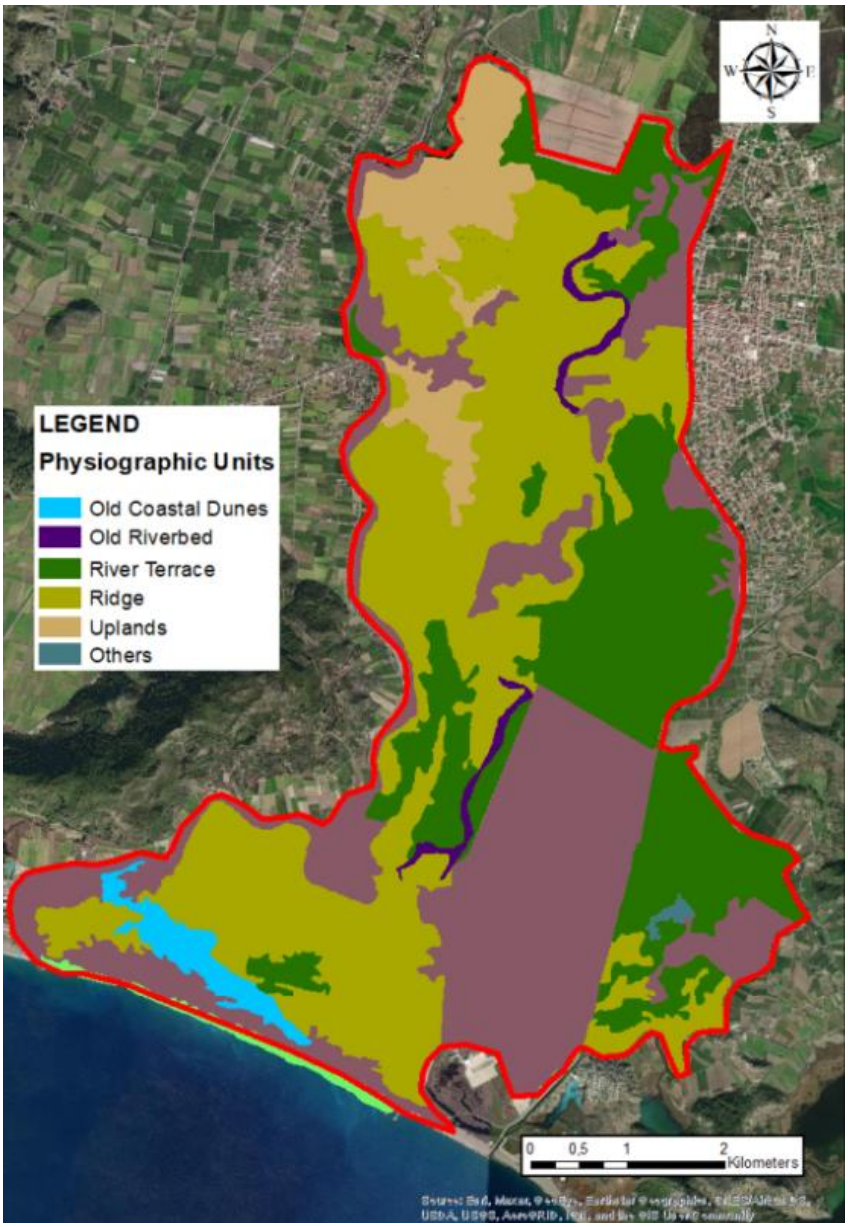


Figure 3. Physiographic units of the study area

Table 2. Physiographic units in the study area

| Physiographic Units | Area (da) | Area (%) |
|---|---|---|
| Old Coastal Dunes | 792 | 2 |
| Old Riverbed | 629 | 1 |
| Ridge | 17291 | 40 |
| River Terrace | 10776 | 25 |
| Uplands | 2973 | 7 |
| Others | 10627 | 25 |
| Total Area | 43088 | 100 |

### Top Soil Texture

When the textures of the surface horizons of the soils are examined, it is seen that there are medium and fine textured soils. Top soil textures are clay, silty clay ones are heavy; Loam, clay loam, silty clay loam and sandy loam are evaluated as medium textured. With this approach, the top textures of Dalaman TİGEM soils generally have a medium texture. While 47% of the study area has a medium texture, 28% has a heavy texture. Since 25% of the study area is used for various purposes such as cattle breeding facility, airport, farm building, there is no information about the soil structure. Surface soil texture classes and distributions obtained by database inquiries are given in Table 3; The distribution map of this data is given in Figure 4.

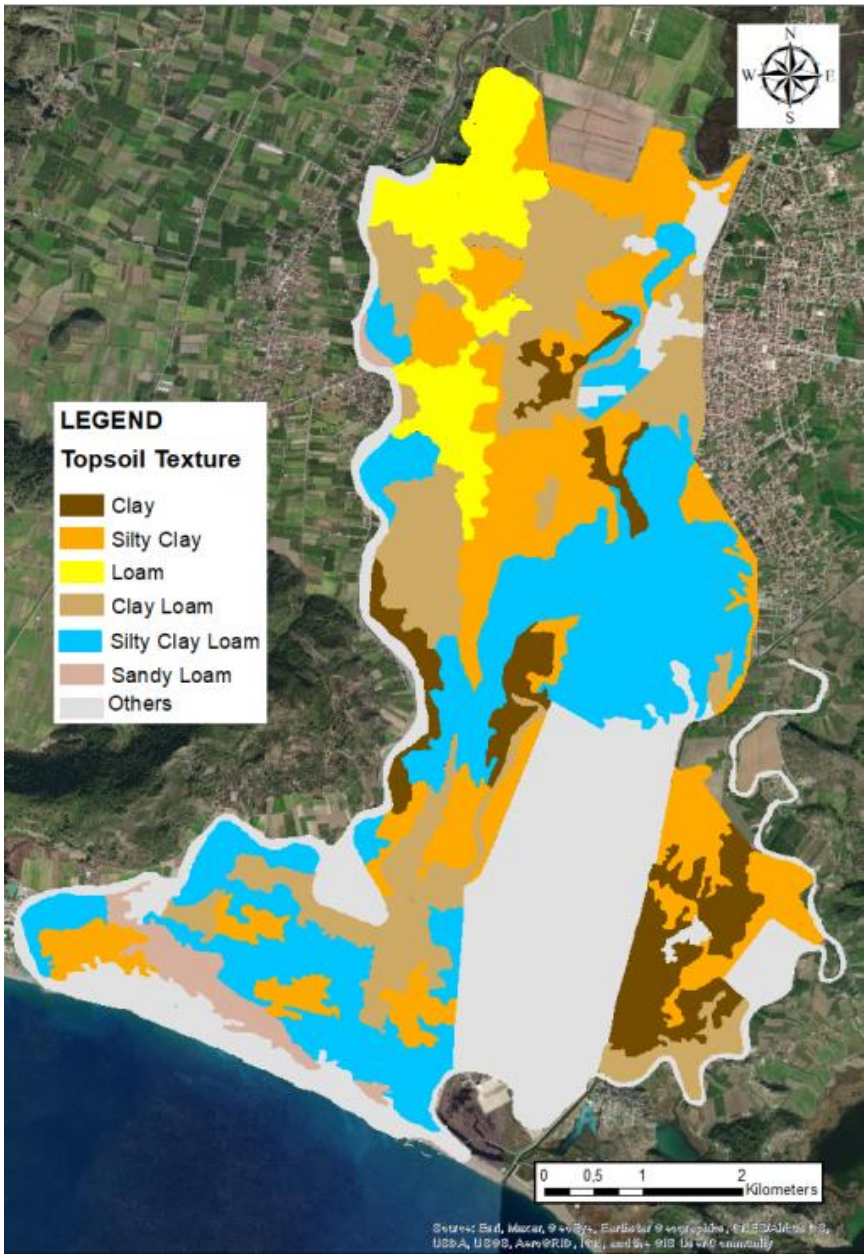


Figure 4. Top soil texture map of the study area

Table 3. Top soil texture of the study area

| Top soil texture | Area (da) | Area (%) |
|---|---|---|
| Clay, Silty Clay | 12072 | 28 |
| Loam, Clay Loam, Silty Clay Loam, Sandy Loam | 20389 | 47 |
| Others | 10627 | 25 |
| Total Area | 43088 | 100 |

## Erosion

Soil erosion is one of the important problems in the study area. There are severe erosion effects in some lands spreading in the higher parts of Gökdağ and Aladağ series in the northwest. These lands have both steep and shallow. However, these lands constitute only %4 of the study area. There is no erosion problem in a significant part of it. Information on the erosion distribution obtained in the GIS is given in Table 4.

Table 4. Erosion levels of the study area

| Erosion | Area (da) | Area (%) |
|---|---|---|
| Severe | 1865 | 4 |
| No erosion problem | 30596 | 71 |
| Others | 10627 | 25 |
| Total Areas | 43088 | 100 |

## Drainage

According to the detailed soil survey report; there are good, poor, bad, very bad and excessive drainage classes in the study area. While most of the lands have good drainage, an area of 6304 da (15%) has poor, very bad and excessive drainage conditions (Table 5). Excessive drainage conditions, which are define as one of the worst drainage classes are especially observed in Söğüt series soils. The soils of series extend in a narrow strip on the coast. There is not enough soil formation for agriculture on these lands, which have sparse scrub as natural vegetation. The drainage map of the enterprise land created in the GIS is given in Figure 5.

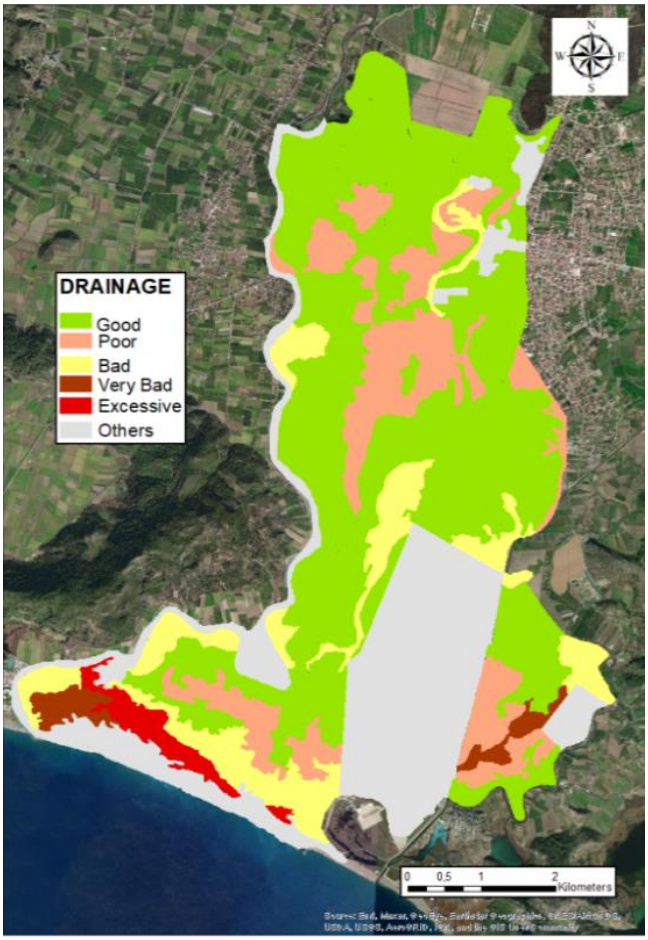


Figure 5. Drainage map of the study area

Table 5. Drainage class of the study area

| Drainage Classes | Area (da) | Area (%) |
|---|---|---|
| Good | 19685 | 46 |
| Poor | 6472 | 15 |
| Bad | 4730 | 11 |
| Very Bad | 782 | 2 |
| Excessive | 792 | 2 |
| Others | 10627 | 25 |
| Total Areas | 43088 | 100 |

## General Properties of Surface Horizons

Except for Gökdağ series, the general features of the 6 series are given together. When the report of the study area was examined, it was seen that soil sampling was not made for identification purposes from Gökdağ series, and therefore its physical and chemical properties were not determined. For this reason, the data were evaluated over 6 series in the study area. The classifications of the properties of the soils have been evaluated within the framework of the Soil and Land Classification Standards Technical Instruction and Relevant Legislation" (Anonymous, 2008).

## pH

The pH of the surface horizons of the soils varies between 7.65 and 8.75. While the lowest pH belongs to Adaköy series soils, the highest pH belongs to Dalaman and Camikırı series. While the Adaköy, Söğüt, Eskiköy and Havaalanı series soils, which are in the light alkali class, are distributed in a total of 19384 decares, the Dalaman and Camikırı series, which have a strongly alkaline surface horizon, are located in the land at 9803 decares (Table 6).

Table 6. pH of surface soils of study area

| Surface Soil pH | Series | Area (da) | Class |
|---|---|---|---|
| 7.65 | Adaköy | 1826 | Slightly Alkaline |
| 7.80 | Söğüt | 492 | Slightly Alkaline |
| 7.85 | Eskiköy | 8118 | Slightly Alkaline |
| 7.90 | Havaalanı | 8950 | Slightly Alkaline |
| 8.75 | Dalaman-Camikırı | 9803 | Strong Alkaline |

## Salinity

Salinity levels of the study area vary between 0.05% and 0.18%. Lowest salinity is in Eskiköy, Dalaman and Adaköy series whereas, the highest salinity is observed in Söğüt series soils. There are salty plants in some of the lands located in the south and southeast parts of the study area. There are also areas where there are salt deposits in places. While the soils defined in the salt-free class are 28697 decares, the lightly salted lands are 492 decares (Table 7).

Table 7. Salinity clases of the study area

| Salinity Level (%) | Series | Area (da) | Class |
|---|---|---|---|
| 0.05 | Eskiköy, Dalaman, Adaköy | 19118 | Saltless |
| 0.07 | Havaalanı | 8950 | Saltless |
| 0.10 | Camikırı | 629 | Saltless |
| 0.18 | Söğüt | 492 | Lightly Salted |

## Cation Exchange Capacity (CEC)

Cation Exchange Capacity (CEC), one of the most important chemical indicators of soils, is also considered as one of the fertility indicators of the soil. In the study area, it can be seen that the CEC ranges between 7.60 and 28.20 me/100gr (Table 8). It belongs to the lowest CEC Söğüt series in the study area. The coarse texture of these soils is a factor in the low CEC level. On the other hand, Eskiköy series are the soils with the highest CEC level in the study area.

Table 8. CEC levels of the study area

| CEC (cmolc/kg) | Series | Area (da) |
|---|---|---|
| 7.60 | Söğüt | 492 |
| 18.40 | Havaalanı | 8950 |
| 21.72 | Dalaman | 9174 |
| 22.20 | Camikırı, Adaköy | 2456 |
| 28.20 | Eskiköy | 8118 |

## Soil Organic Matter (SOM)

Generally, low-medium organic matter levels are observed in continuously cultivated lands. This also applies to the farm land. The organic level in the study area is between 1.24% and 3.20%. Table 9 gives the organic matter level of the series. Accordingly, only Dalaman and Camikırı series contain rich organic matter. In terms of organic matter, poor areas are 8950 da, middle areas are 10436 da and rich areas are 9803 da.

Table 9. Soil organic matter classes of the study area

| SOM (%) | Series | Area (da) | Class |
|---|---|---|---|
| 1.24 | Havaalanı | 8950 | Low |
| 1.89 | Adaköy | 1826 | Medium |
| 2.09 | Söğüt | 492 | Medium |
| 2.16 | Eskiköy | 8118 | Medium |
| 2.61 | Dalaman | 9174 | High |
| 3.20 | Camikırı | 629 | High |

## Calcium Carbonate ($CaCO_3^-$)

$CaCO_3^-$ level is high in all soil series of the soils except Gökdağ series. The high level of lime in the whole profile sometimes reflects negatively on plant production. High lime level negatively affects the intake of plant nutrients, especially useful phosphorus. When analyzed on the basis of series, Dalaman and Eskiköy series are classified as calcareous, while Camikırı, Adaköy, Söğüt and Havaalanı series are classified as very calcareous. 17291 da of the enterprise lands are calcareous; 11897 da is very calcareous (Table 10).

Table 10. Calcium carbonate classes of the study area

| $CaCO_3^-$(%) | Series | Area (da) | Class |
|---|---|---|---|
| 10.66 | Dalaman | 9174 | High |
| 12.74 | Eskiköy | 8118 | High |
| 16.23 | Camikırı | 629 | Very High |
| 17.39 | Adaköy | 1826 | Very High |
| 20.11 | Söğüt | 492 | Very High |
| 21.10 | Havaalanı | 8950 | Very High |

## Conclusion

In this study, it is aimed to evaluate the Dalaman Agricultural Enterprise Lands in GIS and to create maps for general soil properties. There are 7 series in the enterprise. However, since no soil could be taken from Gökdağ series for sampling, evaluations were made about the characteristics of the 6 series soil and their maps were produced. Dalaman and Havaalanı series are the most widespread in the study area. Although the soils of both series have similar characteristics, they are distinguished from other series by the presence of silty clay loam bands under the surface in Havaalanı series. Dalaman Stream had a significant impact on the formation of soils. Dalaman Stream has an effect on the formation of the most widespread river ridge and river terrace physiographic units. In the inquiries made in the GIS, it was determined that these two physiographic units spread over 65% of the total area. In terms of soil texture, 47% of the soils have medium texture, while 28% have fine texture. There is no significant erosion problem in the study area. However, it should be taken into account that 4% of the study area has severe erosion. Lands generally have good drainage. However, it was determined that there was a drainage problem in the land in 6304. The study area generally consists of flat or near-flat lands. The general characteristics of the land were digitized and various inquiries were made with the detailed soil map of the enterprise land, which was transferred to the computer and digitized with ArcGIS software. In addition to this, various thematic maps were created, allowing users to interpret soil characteristics in an easier and more understandable way. This is the most important feature of GIS software. Soil maps in print are difficult to understand and interpreters by other researchers than soil experts. However, with the production and visualization of digitized and various thematic maps, the intelligibility of these maps is increased. A lot of information about agricultural production can be obtained by interpreting soil properties. In addition to determining the

appropriate product variety, management such as tillage, fertilization and irrigation, as well as the suitability of soils for various uses are also important for sustainable agriculture. It is stated that some soil properties are invariant. However, some dynamic properties of soils such as salinity, lime, surface pH, drainage may also change depending on usage conditions. For this reason, it is important to transfer the obtained maps to digital media and to update the changes in the soil and land periodically in the database. It is recommended to update the maps obtained with this study with revision soil surveys, and to make inquiries by processing the changes in the enterprise land into the database.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

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# Determining the future trends of safflower plant in Türkiye

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## Abstract

The aim of this study is to reveal the current situation of safflower in Türkiye, to make future forecasts about safflower planting area, production quantity, yield and producer prices. The Double Exponential Smoothing Method was used in time series analysis for future forecasts. The time series used were obtained from FAO and TUIK statistics. Five-year (2022-2026) future projections were made for the planting area, production quantity, and yield using safflower data between 1988-2021. For the producer prices, the data between the years 2004-2021 were used. According to the results of the study, it is forecasted that in the next 5 years, there will be a reduction average of 54 decares of safflower in the planting areas and an average of 515 tons of reduction in the production quantity each year. Producer prices are expected to increase by an average of 20% per year. Although it is predicted that there will be a very small increase in yield for each year, it is predicted that there will be a 2.5% yield increase at the end of 5 years. In order to prevent the decrease in safflower production and to increase production, it is recommended to provide purchase guarantee to producers, to support entrepreneurs, to carry out studies on modern cultivation practices, marketing and promotion.

## Keywords

Safflower, Future Forecast, Double Exponential Smoothing Method, Türkiye

## Introduction

Safflower (Carthamus tinctorius L.) is a plant species considered to have been domesticated in the Middle East. It is assumed that it was formerly cultivated for food coloring and fabric dyeing with the pigments found in its flowers (Knowles, 1980). In addition to its use in medicine, it is also used as bird seed (Dajue and Mündel, 1996; Ekin, 2005; Bergman et al., 2007, Emongor, 2010). New uses include special types of edible oils that improve human nutrition (Velasco and Fernández-Maryinnez, 2004), biofuel (Bergman and Flynn, 2009), easy isolation of oleosine proteins from safflower seed, and the production of transgenic pharmaceuticals (McPherson et al., 2004; Mündel and Bergman, 2009).

Safflower has been adapted to many semi-arid regions of the world (Mündel et al., 1997). Knowles (1989) described three general climatic areas to which safflower is most adapted: 1) areas like south central India, where the crop is autumn-sown in October or November into heavy soils moist from summer rains or irrigated and harvested in the dry spring; 2) areas that have a Mediterranean type climate like Australia, Mexico, Spain, Middle East countries, California in the USA, where typically the winters are moist and the summers are dry; and 3) areas that have a climate similar to the Northern Great Plains of the USA and Canada, where safflower is sown in the spring and harvested in the dry fall months of September and October (Emongor et al, 2017). In Table 1, the planting area (ha), production quantity (tons), and yield (hg/ha) of the countries that have a significant share in the world safflower production in 2020 are given.

While Kazakhistan ranked first among 13 countries in safflower seed production in 2020, this production quantity constitutes 34.72% of the total safflower seed production in the world. The first five countries (Kazakhistan, Russian Federation, India, United States of America, and Mexico) constitute 79.81% of the total production.

There are some publications explaining the reasons for the increasing popularity of safflower in the world. One of these is the potential medical benefit advocated by Gomashe et al. in 2021. According to Gomashe at al. (2021) safflower has pharmaceutical potential for the treatment of male and female infertility, cardiovascular diseases, reduction in the blood glucose level, treatment of cancer and reduction in the plasma cholesterol level, etc.

Safflower silage has shown potential as an alternative forage crop in semi-arid regions (Weinberg et al., 2002) and vegetal safflower can be used as a substitute for grain silages in the diet of high-yielding dairy cows without affecting their milk performance. Safflower pastures are often argued to be more than adequate for ruminants with moderate requirements for rangeland quality (Landau et al., 2005). There are many publications asserting that safflower is a non-polluting alternative fuel produced from renewable resources, whose chemical and physical properties are very similar to petroleum diesel fuel, with its use as biodiesel (Mishra & Goswami, 2017; Demirbas, 2007; Eryilmaz, at al., 2016). In this way, supplying some of the energy consumed worldwide with biodiesel may explain the increasing interest in safflower (Mihaela ve ark, 2013). Although the oilseed plant widely cultivated in Türkiye stands out as sunflower, safflower farming emerges as an alternative oilseed plant for Türkiye (Kolsarici ve ark.,2005, Ilkdogan & Olhan, 2012). It is thought that safflower cultivation was first practiced in Türkiye in the Marmara Region between 1940-1945 by immigrants from Bulgaria. (Babaoglu 2006). When we look at the quantity of safflower production between 2010-2021 and the planting area in parallel, an increasing trend is observed until 2015, while a rapid decline is observed in recent years (Table 2). Ilkdogan & Olhan, (2012) explained this decrease with the preference of transgenic species (soybean, rapeseed and cotton) worldwide. According to 2021 data, the 5 provinces where 65.5% of safflower production is made in Türkiye are Ankara (3974 tons), Aksaray (2232 tons), Kayseri (2201 tons), Konya (1340 tons) and Nevşehir (877 tons), respectively.

Table 1. World safflower planting area, production, and yield quantity in 2020 by country

| Country | Planting Area (ha) | Production Quantity (ton) | Yield (hg/ha) |
|---|---|---|---|
| Kazakhstan | 315.177 | 226.739 | 7.194 |
| Russian Federation | 174.974 | 96.636 | 5.523 |
| India | 85.475 | 44.000 | 5.148 |
| United States of America | 51.270 | 67.040 | 13.076 |
| Mexico | 50.414 | 86.793 | 17.216 |
| Argentina | 27.349 | 22.565 | 8.251 |
| United Republic of Tanzania | 25.170 | 13.721 | 5.451 |
| China, mainland | 22.724 | 33.404 | 14.700 |
| Uzbekistan | 18.324 | 8.885 | 4.849 |
| Türkiye | 15.114 | 21.325 | 14.109 |
| Kyrgyzstan | 9.836 | 9.870 | 10.035 |
| Ethiopia | 7.442 | 9.349 | 12.562 |
| Australia | 6.195 | 3.602 | 5.814 |
| Iran (Islamic Republic of) | 3.568 | 4.701 | 13.175 |
| Tajikistan | 3.438 | 4.293 | 12.487 |

Source: FAO, 2022

Table 2. Safflower planting area, production quantity, and yield in Türkiye

| Year | Planting Area (da) | Production Quantity (ton) | Yield (kg/da) | Producer Prices (TL) |
|---|---|---|---|---|
| 2010 | 135.000 | 26.000 | 193 | 0,54 |
| 2011 | 131.668 | 18.228 | 138 | 0,61 |
| 2012 | 155.918 | 19.500 | 128 | 0,64 |
| 2013 | 292.920 | 45.000 | 154 | 0,76 |
| 2014 | 443.050 | 62.000 | 140 | 0,79 |
| 2015 | 431.071 | 70.000 | 162 | 0,86 |
| 2016 | 395.710 | 58.000 | 147 | 0,95 |
| 2017 | 273.762 | 50.000 | 183 | 0,97 |
| 2018 | 246.932 | 35.000 | 142 | 1,19 |
| 2019 | 158.601 | 21.883 | 138 | 1,72 |
| 2020 | 151.150 | 21.325 | 141 | 2,41 |
| 2021 | 145.882 | 16.200 | 111 | 3,18 |

Source: TUIK,2022

The most basic agricultural policy instrument applied in Türkiye regarding safflower is the deficiency payment per kg, which has been implemented since 2006, in order to increase the production of this product,

also known as premium support, diesel-fertilizer and domestic certified seed usage support payments. According to the data obtained by Ilkdogan and Olhan, (2012) from the producers engaged in safflower farming in the Central Anatolia Region, 40% of the producers find the agricultural support given to safflower satisfying, 30% because it is a good animal feed, and 20% because it is easy to grow safflower and 10% stated that they continue to plant safflower because they make good profits from safflower farming.

The aim of this study; to observe the changes in the planting area, production quantity, yield and producer prices for safflower cultivation in Türkiye in the coming years, to make forecasts about the future and to offer recommendations in line with these forecasts.

## Material and Method

### Material

In this study, safflower seed, which has an important place in the oil industry in Türkiye, but whose production is not sufficient, has been examined. The quantity of safflower production, planting area, and yield obtained from FAO (Food and Agriculture Organization of the United Nations) and TUIK (Turkish Statistical Institute) for the years 1988-2021 were examined and 5-year estimates were made for these years. As for the production prices, the data were used between the years 2004-2021. The reason for this situation is that the current data sources provide price data until 2004. In addition, the past and current policies about the safflower plant were evaluated in the study.

### Method

The situation in Türkiye's safflower market has been revealed with complementary statistics and the values that the production quantity, planting area, yield and price variables will reach in the next 5 years have been estimated and interpreted. Throughout the study, 34 years time series data covering the years 1988-2021 were used. In the study, Kolmogorov Smirnov test was used to determine whether each variable provides a normal distribution. According to the Kolmogorov Smirnov test, the non-normally distributed variable data was transformed into a normal distribution state by applying a transformation, and then the stationarity of the variable data was examined.

The "Double Exponential Smoothing" method was used to make future estimations of safflower production quantity, planting area, yield and producer prices. This method is preferred because of the last changes, jumps, monitoring of linear trend and exponential correction in the time series. The equations of this method are as follows.

Average: $A_t = \alpha Y_t + (1-\alpha)(A_{t-1} + T_{t-1})$

Current Trend: $CT_t = A_t - A_{t-1}$

Average Trend: $T_t = \beta CT_t + (1-\beta)T_{t-1}$

Prediction: $\hat{Y}_{t+1} = A_t + T_t$

In the equations, $A_t$ is the exponentially corrected mean of the series in the t period, $CT_t$ is the current trend in the t period, $T_t$ is the exponentially corrected trend in the t period, $\hat{Y}_{t+1}$ is the prediction for the next period, α is the correction factor for the mean ranging from 0 to 1, β shows the correction factor for the trend ranging from 0 to 1. In order to start the application of this method, initial estimates for the mean and trend are needed. In this study, very different combinations of α and β were tried and the combination that gave the best prediction was selected (Anonymous, 2016).

## Findings on the Future of Safflower Plant in Türkiye

### Safflower Planting Area Forecast

Time series data between 1988 and 2021 were used in the forecasts made for the planting area of safflower in Türkiye.

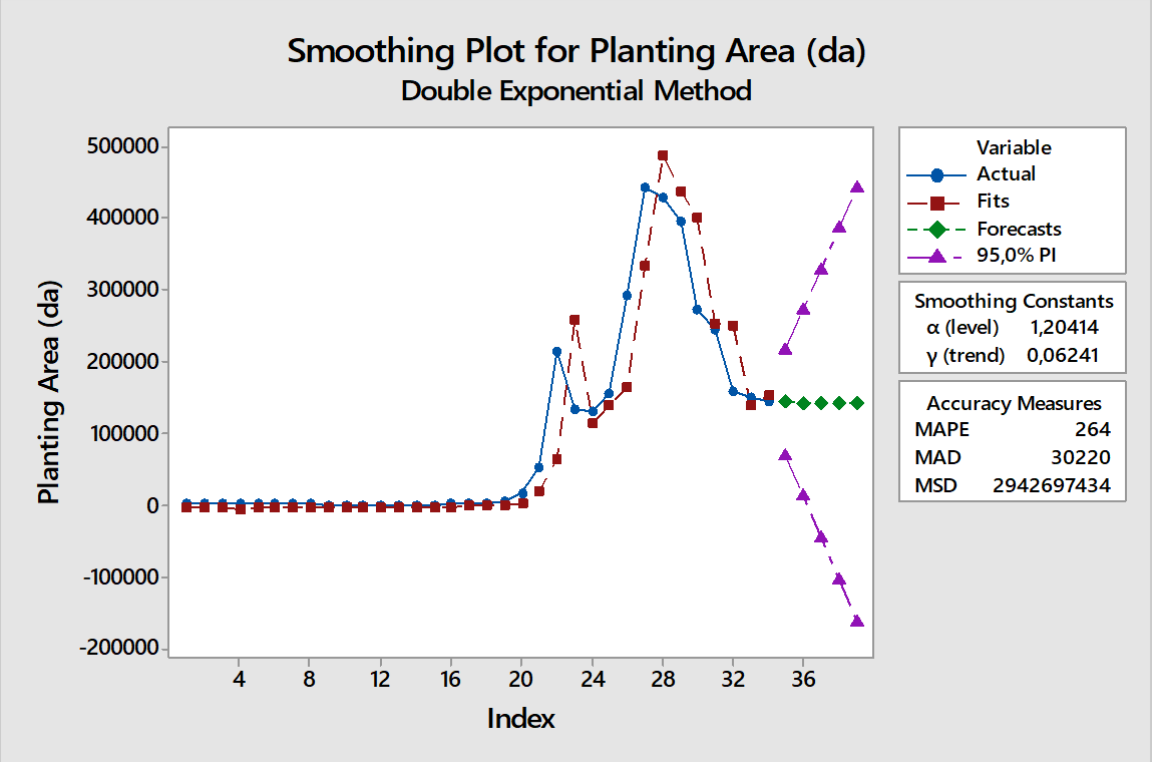


Figure 1. Safflower planting area forecast chart

As can be seen from Figure 1 in the projection made on the safflower planting area of Türkiye, it is seen that the planting areas will decrease gradually, if not very much, in the next 5 years. While there was 143.632 da of safflower planting area in 2022 in Türkiye, it is predicted that it will decrease to 140.950 da in 2026 (Table 3). In the worst case, provided that all conditions remain the same, it is predicted that safflower planting areas will reach the point of extinction (to be replaced by other products) in 2026. The most important reason for the decrease in safflower planting areas and production in Türkiye is the low safflower yield and safflower oil rate, and for these reasons, safflower cannot compete with plants grown under almost the same conditions (Baydar and Kara, 2010).

Table 3. Forecast of safflower planting area in Türkiye for 5 years (da)

| Years | Forecast Value | Maximum | Minimum |
|---|---|---|---|
| 2022 | 143.632 | 217.670 | 69.593 |
| 2023 | 142.961 | 272.781 | 13.142 |
| 2024 | 142.291 | 329.563 | 0 |
| 2025 | 141.621 | 386.845 | 0 |
| 2026 | 140.950 | 444.340 | 0 |

## Safflower Production Quantity Forecast

Time series data between 1988-2021 were used in the forecasts made for the production quantity of safflower plant in Türkiye.

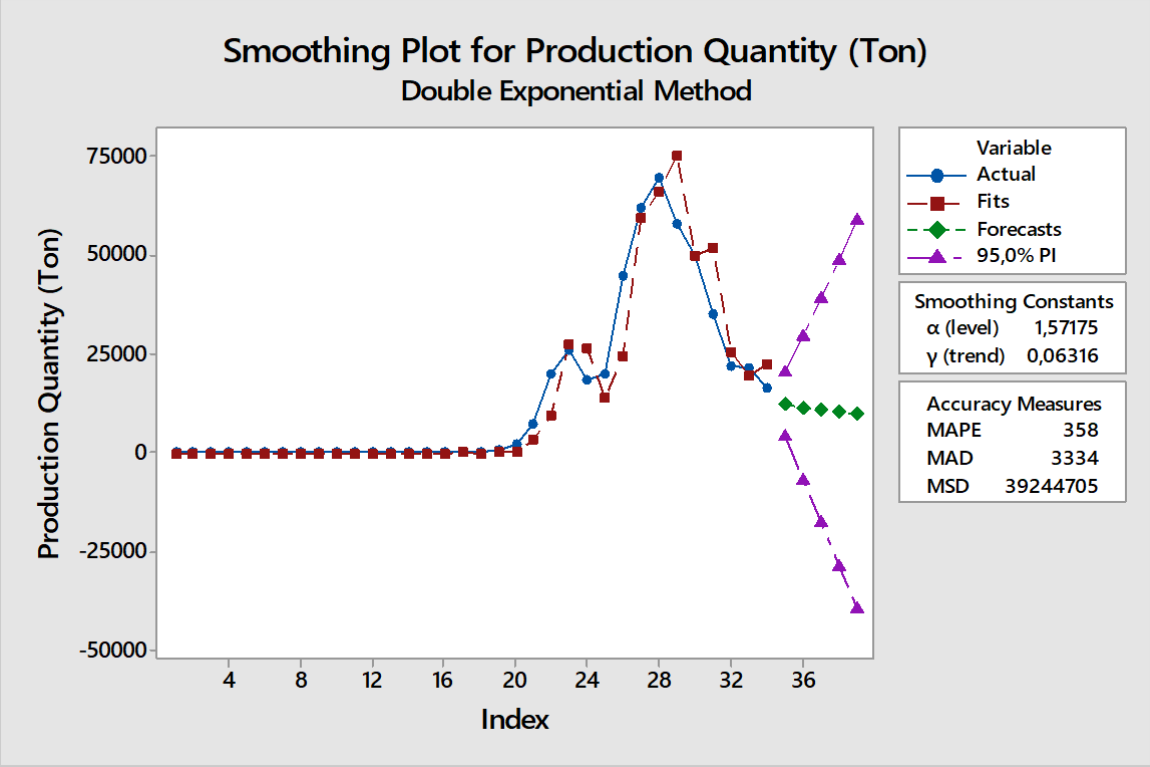


Figure 2. Safflower production quantity forecast chart

In the forecast made on the quantity of safflower production in Türkiye, as can be seen from Figure 2, it is seen that the gradual decrease in the production quantity will continue in connection with the decrease in the planting areas. In Table 4, it is predicted that the quantity of safflower production, which was 12,099 tons in 2022, will decrease to 9,525 tons in 2026. If other conditions remain constant, it is predicted that safflower production may come to a standstill in 2026 in the worst-case scenario. In order to increase safflower production and to provide economic benefits and efficiency, modern cultivation methods should be used, and new varieties with high yield should be developed by using advanced breeding methods (Baydar, Gokmen and Friedt, 2003).

Table 4. Forecast for 5-year safflower production in Türkiye (tons)

| Years | Forecast Value | Maximum | Minimum |
|---|---|---|---|
| 2022 | 12.099 | 20.267 | 3.932 |
| 2023 | 11.456 | 29.845 | 0 |
| 2024 | 10.812 | 39.499 | 0 |
| 2025 | 10.169 | 49.170 | 0 |
| 2026 | 9.525 | 58.846 | 0 |

### Safflower Yield Forecast

Time series data between 1988-2021 were used in the forecasts made for the yield of safflower plant in Türkiye.

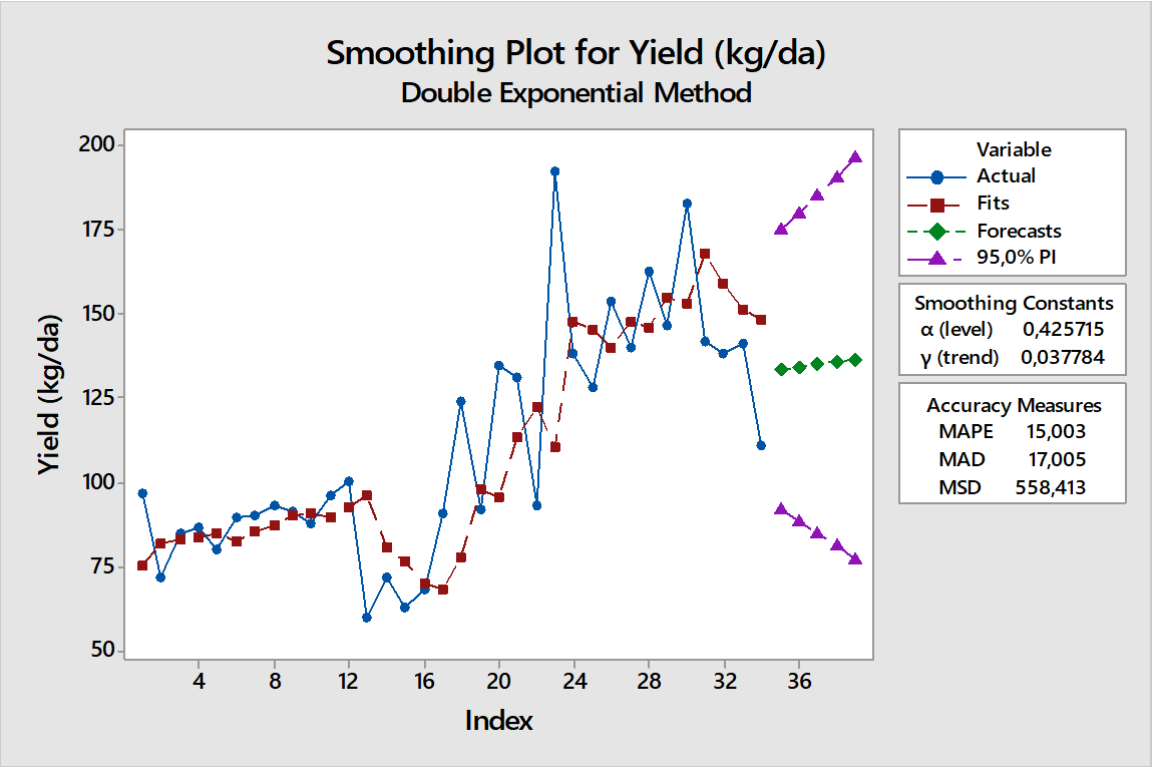


Figure 3. Safflower yield forecast chart

Forecast of safflower yield in Türkiye as seen in Figure 3, it is predicted that yield will increase contrary to the estimations of planting area and production quantity. While safflower yield is 133,4 kg per decare in 2022, it is predicted that it will be 136,7 kg per decare in 2026 (Table 5). When the literature studies on the subject are examined; Baydar et al. (2003), Baydar and Kara (2010), Baydar and Erbas (2016) suggested in their studies that the yield of safflower is not sufficient and that high yielding varieties should be developed. While there are currently 15 registered safflower varieties in Türkiye, 6 of them were registered in 2019 and their yield values are above the world yield averages. (TTSM, 2021). Aslan et al. (2019) also stated in their study on the examination of newly developed safflower varieties that the yield of new varieties of safflower is high. In addition, the yields of 5 countries (Kazakhistan: 72 kg/da, Russia: 55 kg/da, America: 130 kg/da, Mexico: 172 kg/da and India: 51 kg/da) that produced 80% of world safflower production in 2020 when examined, it is seen that Türkiye ranks first in safflower yield (FAO, 2021). For this reason, it is understood that the decrease in safflower planting area is not caused by low yield and the problem of low yield of safflower is resolved. While yield climatic conditions, precipitation regime, technological developments, etc. depending on factors such as, improvements in production techniques, improvement in the economic conditions of the producers, and the use of efficient varieties also have an effect on increasing the yield (Bolat et al. 2017). With the use of productive safflower varieties developed in Türkiye in recent years, the yield per unit area can be increased even more. As seen in Table 5, if all conditions remain the same, it is predicted that the highest safflower yield will be 196,7 kg per decare and the lowest 76,7 kg per decare in 2026.

Table 5. Forecast of safflower yield in Türkiye for 5 years (kg/da)

| Years | Forecast Value | Maximum | Minimum |
|---|---|---|---|
| 2022 | 133,4 | 175,0 | 91,7 |
| 2023 | 134,2 | 179,9 | 88,5 |
| 2024 | 135,1 | 185,2 | 84,9 |
| 2025 | 135,9 | 190,8 | 80,9 |
| 2026 | 136,7 | 196,7 | 76,7 |

### Safflower Price Forecast

In the forecasts made on the producer prices of the safflower plant in Türkiye, nominal price (TL: Turkish Liras) data for the years 2004-2021 were used. Different from the planting area, production quantity and yield, the data between the years 2004-2021 were used in the production prices. The reason for this situation is that the current data sources provide price data until 2004.

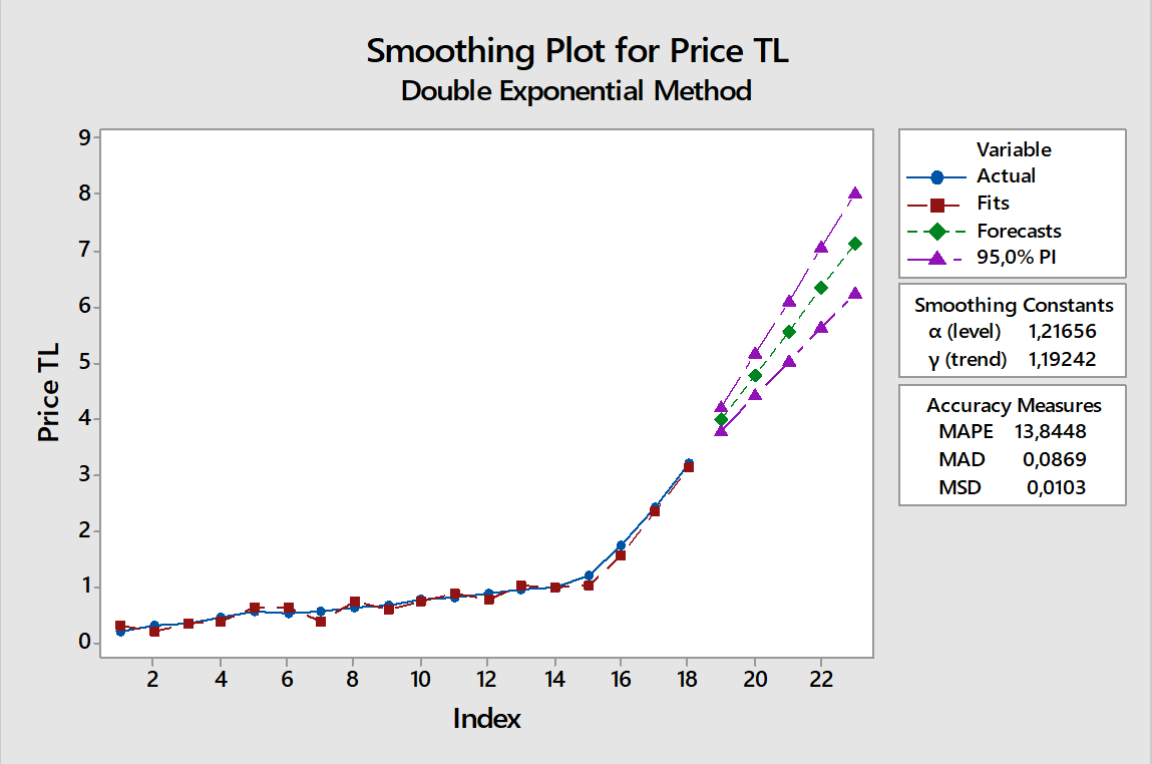


Figure 4. Safflower nominal producer price forecast chart

In the forecast made for safflower producer prices in Türkiye, it is predicted that there will be an increase in prices over the years, as seen in Figure 4. It is predicted that the producer price of safflower will reach 3,97 TL/kg in 2022 and 7,11 TL/kg in 2026 (Table 6). It is thought that safflower production will decrease further in the coming years due to the increase in producer costs. In addition to the decrease in production, the increased production cost has also been effective on the increase in safflower prices (Bolat et al, 2017).

Table 6. Forecast for 5-year nominal safflower producer prices in Türkiye (TL/kg)

| Years | Forecast Value | Maximum | Minimum |
|---|---|---|---|
| 2022 | 3,97 | 4,19 | 3,76 |
| 2023 | 4,76 | 5,14 | 4,38 |
| 2024 | 5,54 | 6,09 | 5,00 |
| 2025 | 6,33 | 7,04 | 5,61 |
| 2026 | 7,11 | 8,00 | 6,23 |

## Result

Safflower is a very important plant in terms of contributing to the biodiesel, dye, oil and feed industry, being easily adapted to the application of rotation, being effective in reducing fallow areas, being able to grow in different types of soil, not needing special equipment in the cultivation process, and contributing to the sustainability of agricultural production and employment.

While safflower has been included in the scope of support by the Ministry of Agriculture and Rural Affairs (former Republic of Türkiye Ministry of Agriculture and Foresty) since 2005 in Türkiye, product support per kilogram has been given, in 2006 deficiency payment support has been started and continues to increase every year. As a result of these supports, gradual increases were observed in production and reached the highest safflower production level in 2014-2015. After the years that reached the highest level, there was a decrease in production due to problems such as yield, oil capacity, marketing, and inability to compete easily with other products.

In recent years, the decrease in safflower production has continued. In the period of 2014-2021, safflower planting area 67% and production quantity 74% decreased. In addition, producer prices increased more than 3 times in the same period. According to the 5-year forecast results made in the study, it is predicted that there will be a decrease in the planting area and production quantity, while it is predicted that the yield and producer price will increase.

Safflower; in the next 5 years, it is predicted that there will be an average of 54 decares (It was calculated by taking the 2022 forecast value difference from the 2026 forecast value and dividing it by the number of forecast years) decrease in planting areas and an average of 515* tons of reduction in production each year. Producer prices are expected to increase by an average of 20% per year. Although it is estimated that there will be a very small increase in yield for each year, in total, there will be a 2.5% yield increase at the end of 5 years.

As a result of the examination of previous studies on the subject and research findings, the things to be done in order to prevent the decrease in safflower production in the future can be listed as follows;

Purchase guarantee should be provided for safflower production

Safflower should be evaluated within the scope of intervention purchases.

Safflower purchase price should be increased

R&D studies should be carried out to increase safflower production.

Entrepreneurs should be given incentives for the establishment of the safflower industry

Technical support should be given to farmers for safflower cultivation and modern cultivation should be ensured.

Facilitation should be provided in the supply of safflower seeds

By emphasizing the importance and benefits of safflower oil, it should be promoted on a consumer basis and demand should be created.

Safflower cultivation has also an important place in the feed industry. Therefore, it should be encouraged by goverment considering that factor.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript.

All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Microsatellite analysis in some watermelon (*Citrullus lanatus*) genotypes

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## Abstract

Conservation of genetic resources is essential for the continuation of future crop production. Watermelon (Citrul-lus lanatus), a member of Cucurbitaceae, is widely distributed in tropical and subtropical regions. The aim of this study was to reveal the genetic relationships with the help of microsatellite markers in a watermelon collection free of unnecessary repetitions, and to determine the success of SSR (Simple Sequence Repeats) primers developed in cucurbits. In this study, 96 watermelon genotypes with good agronomic characteristics were used among the geno types collected from different regions of Turkey and purified up to the S4-S6 (self-pollination) stage. In the study, 33 SSR primer pairs were used to determine the genetic relationship between watermelon genotypes. In the study, a total of 67 bands were obtained with SSR primers. As a result of UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) analysis, genotypes showed similarity at the level of 0.84-1.00. The number of alleles detected per primer varied between 1 and 6. In terms of the total number of alleles obtained, CMCT44 (5 units) and Cgb4767 (6 units) loci produced the most alleles. Primers with high polymorphism rate and allele excess were determined, and the possibilities for use in genetic stability analyses, variety differentiation and other genetic analyses were determined.

## Keywords

*Citrullus lanatus*, watermelon, SSR, microsatellite, genetic characterization

## Introduction

Watermelon is a member of the Cucurbitaceae family, which includes many commercial species such as melon, cucumber, squash, gourd, and pumpkins. Watermelon originating from South Africa is widespread in subtropical and tropical regions (Düzyaman, 2013). Watermelon is an important species with economic value in the Cucurbitaceae family, and its fruits differ considerably in terms of size and shape. Watermelons, which have seeded and seedless varieties, are the species whose leaves are highly fragmented (Solmaz et al., 2010). Watermelon cultivation is carried out in a very wide area in the world. As of 2019, watermelon production in the world is 100.4 million tons (Anonymous, 2021). China and Turkey lead the world in watermelon production.

Watermelon contains various vitamins (A, B, C and E), carotenoids (lycopene and beta-carotene), amino acids and some phenolic compounds (Tlili et al., 2009). Due to the lycopene, it contains, watermelon is a good antioxidant and is known to reduce the risk of prostate, stomach, and pancreatic cancer in humans (Collins et al., 2006). It has been stated that the lycopene content (23-72 µg/g/fresh weight) in watermelon is higher than other vegetables and fruits. It is very important to genetically improve and protect vegetables that stand out with their nutritional value and agronomic properties. Conservation of plant genetic resources is very important for future breeding studies. Plant gene resources are faced with the threat of decrease and loss due to environmental and other effects in the regions where they are located. Conservation of genetic

resources is essential for the continuity of plant production. Since the number of varieties in agricultural products is constantly increasing, morphological markers are insufficient to detect the differences between varieties. Therefore, molecular markers should also be used to protect genetic resources (Lombard et al., 2001).

In-plant genetics and breeding, genetic markers are generally used in selection, variety identification and genome mapping. After the discovery of the PCR reaction, a wide variety of molecular marker techniques have been developed for mapping, genetic labelling, detection of different gene regions, phylogenetic analysis, genetic diversity studies and Marker Assisted Selection (MAS) studies. Microsatellites or SSRs, consist of sequentially repeated 2-6 nucleotide groups scattered throughout eukaryotic genomes. Among the markers, SSR markers are preferred because of their cost, simplicity, and efficiency (Powel et al., 1996). Since SSRs are highly polymorphic, they give a lot of information to plants. In addition, it is widely used because it gives a codominant marker and has the ease of PCR (Röder et al., 1995). Genetic studies have been successfully carried out in plant species using different molecular marker techniques (Coskun et al., 2017; Karaman et al., 2018; Uzun et al., 2020; Aslan et al., 2021; Morilipınar et al., 2021; Kırac et al., 2022). Genetic characterization studies were performed using SSR markers in different watermelon genotypes (Guerra-Sanz, 2002; Solmaz, 2010; Zhang et al., 2012; Gama et al., 2013; Kwon, 2013; Nantoume et al., 2013; Kong et al., 2014; Lu et al., 2018).

This study, it is aimed to reveal the molecular characterization of watermelon, which has a rich genetic pool, and the genetic relationship between different watermelon genotypes. The main purpose of this study is to perform microsatellite marker analysis in the core watermelon collection free of unnecessary repetitions. This study, it was aimed to optimize the primers and determine the allele sizes on the watermelon lines in the Turkish watermelon seed collection by using the SSR marker technique.

## Materials and Methods

In this study, genotypes collected from different regions of Turkey by Çukurova University Faculty of Agriculture, Department of Horticulture and purified up to S4-S6 grade were used. Among 250 watermelon lines with good agronomic characteristics, 96 genotypes selected from the previous project were used. These genotypes were selected from among those that were found to be the most genetically different from each other. Some of these genotypes are commercial varieties (35 Sugar baby, 235 Charleston Gray Seminis USA, 238 Dixilee North caroline USA, 365 China, G11 DIMA 4B Hungary and G12 Gyulavari Hungary). Other genotypes originate from Turkey.

DNA isolations were made, and PCR studies were carried out. Equal amounts of formamide loading buffer containing 10 mM EDTA (pH 8.0), 95% formamide, 0.025% bromophenol blue and 0.025% xylene cyanol were added to each tube containing the amplification product. PCR products were loaded on a 30% polyacrylamide gel (Long Ranger, FMC Biozym, Hessisch Oldendorf, Germany) and visualized on the 4300 DNA Analyzer (Li-Cor). M13 reverse (GGATAACAATTTCACACGG) or M13 forward (CACGACGTTGTAAAACGAC) primers were added to the 5' end of the synthetically prepared SSR Forward primers (700 or 800 nm wavelength). Data were analyzed using NTSYS program, UPGMA dendrogram was produced and PCA analyses were performed.

## Results and Discussion

In this study, watermelon genotypes belonging to *C. lanatus* var. *lanatus* species, most of which have different geographical origins in Turkey and commercial cultivars were used. In this study, genetic characterization studies were performed on 96 watermelon genotypes with 33 SSR primers showing amplification. A total of 67 bands were obtained and 16 primers (including primers CMTA170a, Cgb4767, CSJCT641 and CSJCT435) showed high polymorphism. In terms of the total number of alleles obtained, CMCT44 (5 units) and Cgb4767 (6 units) loci produced the most alleles (Table 1). In terms of allele sizes detected with the 33 SSR primers used in this study, which produced scoreable bands, the largest allele was obtained from the CSJCT781 locus (330 bp), while the smallest allele was obtained from the CMTA170a (77 bp) and CSCTTT15a (80 bp) loci.

In the study conducted by Solmaz (2010), the highest locus was found in the Cgb4767 locus with 7 alleles. Also, a study examined by Guerra-Sanz (2002) to determine the allele numbers, the number of alleles obtained from 19 microsatellite primers in *C. lanatus* genotypes was between 1 and 8, while the number of alleles was between 1 and 6. In another studies carried out by Zhang et al. (2012) and number of alleles per locus was found to be between 2 and 7. Nantoume et al. (2013) investigated the genetic differentiation of 134 watermelon genotypes in their study and a total of 397 plants were analyzed with 24 SSR primer pairs and a total of 129 alleles were obtained. In our study, a similar number of alleles per primer was obtained. Considering the obtained polymorphism (100%) rate and when compared with studies investigating genetic diversity in watermelons with SSR markers (Danin-Poleg et al., 2001; Tzitzikas et al., 2009; Solmaz, 2010). It is seen that the number of SSR primers used is sufficient.

According to the obtained UPGMA dendrogram, similarity levels were determined between 0.84 and 1.00. In the dendrogram, two main groups were formed at the 0.85 similarity level between 96 genotypes. Genotypes 313 and 182 in the first main group were found to be 99% similar. In the second main group, both 97- 90 genotypes and 147- 194 genotypes are similar (Figure 1). As a result, in the dendrogram obtained from the SSR analysis data, it was revealed that the watermelons belonging to the *C. lanatus* var. *lanatus* subspecies collected from different regions of Turkey are genetically different from each other. In the second group obtained in the dendrogram, genotypes 2 and 9 were located in a single branch, while the others were clustered in a large group (Figure 1). It was determined that the genetic similarity ratios of the studied watermelon genotypes were divided into different subgroups varying between 0.86 and 1.00, and that neither the origin of the watermelon was collected, nor the morphological characteristics had any effect on the

formation of these subgroups. In the study of Sarı et al. (2007), in which the genetic diversity of watermelons collected from different regions of Turkey was investigated with RAPD markers, they determined that

Table 1. Polymorphism rates of SSR primers

| Locus Name | Sequence Information | Total Number of Bands | Number of Polymorphic Bands | Polymorphism Rate |
|---|---|---|---|---|
| CMCT44F<br>CMCT44R | TCAACTGTCCATTTCTCGCTG<br>CCGTAAAGACGAAAACCCTTC | 5 | 5 | %100 |
| CMTA170aF<br>CMTA170aR | TTAAATCCCAAAGACATGGCG<br>AGACGAAGGACGGTTAGCTTT | 2 | 2 | %100 |
| CMCT160aF<br>CMCT160aR | GTCTCTCTCCCTTATCTTCCA<br>ACGGTGTTTGGTGTGAGAAG | 1 | 1 | %100 |
| CSTCC813F<br>CSTCC813R | GTTGTGCTCCCCAATAGTTG<br>CACCACTTCTTCCACCGAA | 2 | 1 | %50 |
| CSAT425F<br>CSAT425R | TAGGGCAGGTATTATTTCAG<br>ACGGACTGATTTAGTATAGGC | 2 | 1 | %50 |
| CMGA104F<br>CMGA104F | TTACTGGGTTTTGCCGATTT<br>AATTCCGTATTCAACTCTCC | 1 | 0 | %0 |
| CMCCA145F<br>CMCCA145R | GAGGGAAGGCAGAAACCAAAG<br>GCTACTTTTGTGGTGGTGG | 2 | 2 | %100 |
| CMTC160a+bF<br>CMTC160a+bR | GTCTCTCTCCCTTATCTTCCA<br>GATGGTGCCTTAGTTGTTCCG | 2 | 1 | %50 |
| CSCT335F<br>CSCT335R | CCTTCACTTCCATCTTCATC<br>CGGTCCTTCATTTCATAGAC | 2 | 2 | %100 |
| CMACC146F<br>CMACC146R | CAACCACCGACTACTAAGTC<br>CGACCAAACCCATCCGATAA | 2 | 2 | %100 |
| CMTC168F<br>CMTC168R | ATCATTGGATGTGGGATTCTC<br>ACAGATGGATGAAACCTTAGG | 3 | 3 | %100 |
| CMGA165F<br>CMGA165R | CTTGTTTCGAGACTATGGTG<br>TTCAACTACAGCAAGGTCAGC | 2 | 2 | %100 |
| CMCT505F<br>CMCT505R | GACAGTAATCACCTCATCAAC<br>GGGAATGTAAATTGGATATG | 2 | 2 | %100 |
| CSTA050F<br>CSTA050R | GAATTATGCAGATGGGTCTT<br>CAAGAAGATCAAATGATAGC | 1 | 0 | %0 |
| CMCTT144F<br>CMCTT144R | CAAAAGGTTTCGATTGGTGGG<br>AAATGGTGGGGGTTGAATAGG | 3 | 2 | %66 |
| CMGA172F<br>CMGA172R | CAATCGCAGATACTTCCACG<br>TGCTTGTCCCAACGGTGTCAT | 1 | 0 | %0 |
| CSCTTT15aF<br>CSCTTT15aR | GTTTGATAATGGCGGATTGT<br>GTAGAAATGAAGGTATGGTGG | 1 | 0 | %0 |
| CMTC51F<br>CMTC51R | ATTGGGGTTTCTTTGAGGTGA<br>CCATGTCTAAAAACTCATGTGG | 2 | 2 | %100 |
| CSJCT 674F<br>CSJCT 674R | TGAAAGGAAGGGATGTGATTAGG<br>ACAGGTGGTTAGAGGTTAGAGCTG | 2 | 1 | %50 |
| Cgb4767F<br>Cgb4767R | TTCTCTTCATTCCCCCAAAATC<br>ACGGGTGAGGGAAAACGAG | 6 | 6 | %100 |
| CSJCT 641F<br>CSJCT 641R | GAACAACCCTCCAATTTTGCTC<br>GCCACTTCCATGTCCAAATTC | 3 | 3 | %100 |
| CSJCT 904F<br>CSJCT 904R | GTAGGCCTGAATTTAGGCATGAGA<br>ATATCACACGCTAACTTTGGGTCA | 3 | 2 | %66 |
| ASUW2F<br>ASUW2R | GCTTCGTTGTTGCTGCCGTTG<br>GCATAAAATCACACTCAAAC | 2 | 2 | %100 |
| CI.2-23F<br>CI.2-23R | GAGGCGGAGGAGTTGAGAG<br>ACAAAACAACGAAACCCATAGC | 3 | 3 | %100 |
| CSJCT 662F<br>CSJCT 662R | ACGTCGTAAAACCATCGGAGTC<br>GCTTCCAAGCGTCAAAGGTATC | 1 | 1 | %100 |
| CSJCT 775F<br>CSJCT 775R | TAGGCCTGAATTTAGGCATAGGAGA<br>TTGGGTCATTTGGTGTATCTAACAC | 2 | 1 | %50 |
| ASUW19F<br>ASUW19R | GTGTGTTTTTGCGTGTG<br>GGGCAAATCCAATAATCCAG | 3 | 3 | %100 |
| C.I.2-140F<br>C.I.2-140R | CTTTTTCTTCTGATTTGACTGG<br>ACTGTTTATCCCGACTTCACTA | 1 | 0 | %0 |
| CSJCT 602F<br>CSJCT 602R | GAGCTGAGCCAAGTTATCGTTTTG<br>CAATTGAGGAAGAGGAGTTGGTTC | 1 | 0 | %0 |
| CSJCT 664F<br>CSJCT 664R | AAGTGGGCTCGATTGGAAGA<br>CCGTCGCCTTTCTCAAGTTC | 1 | 0 | %0 |
| CSJCT 781F<br>CSJCT 781R | AAAGAAGATAGGCCTAGAATTTAG<br>GCCCACATATGTCTAAATTGTCA | 1 | 0 | %0 |
| CLG7992F<br>CLG7992R | CTAACGCAATTTGAATCACTCAAA<br>GGTAAAATGAAATCAATTGTGGAA | 1 | 0 | %0 |
| Cgb5009F<br>Cgb5009R | CAGTGGCACCGTCATCTAAAG<br>AGTGGGGGATTCTCTTCCTAAG | 1 | 0 | %0 |
| TOTAL | | 67 | 53 | %79 |

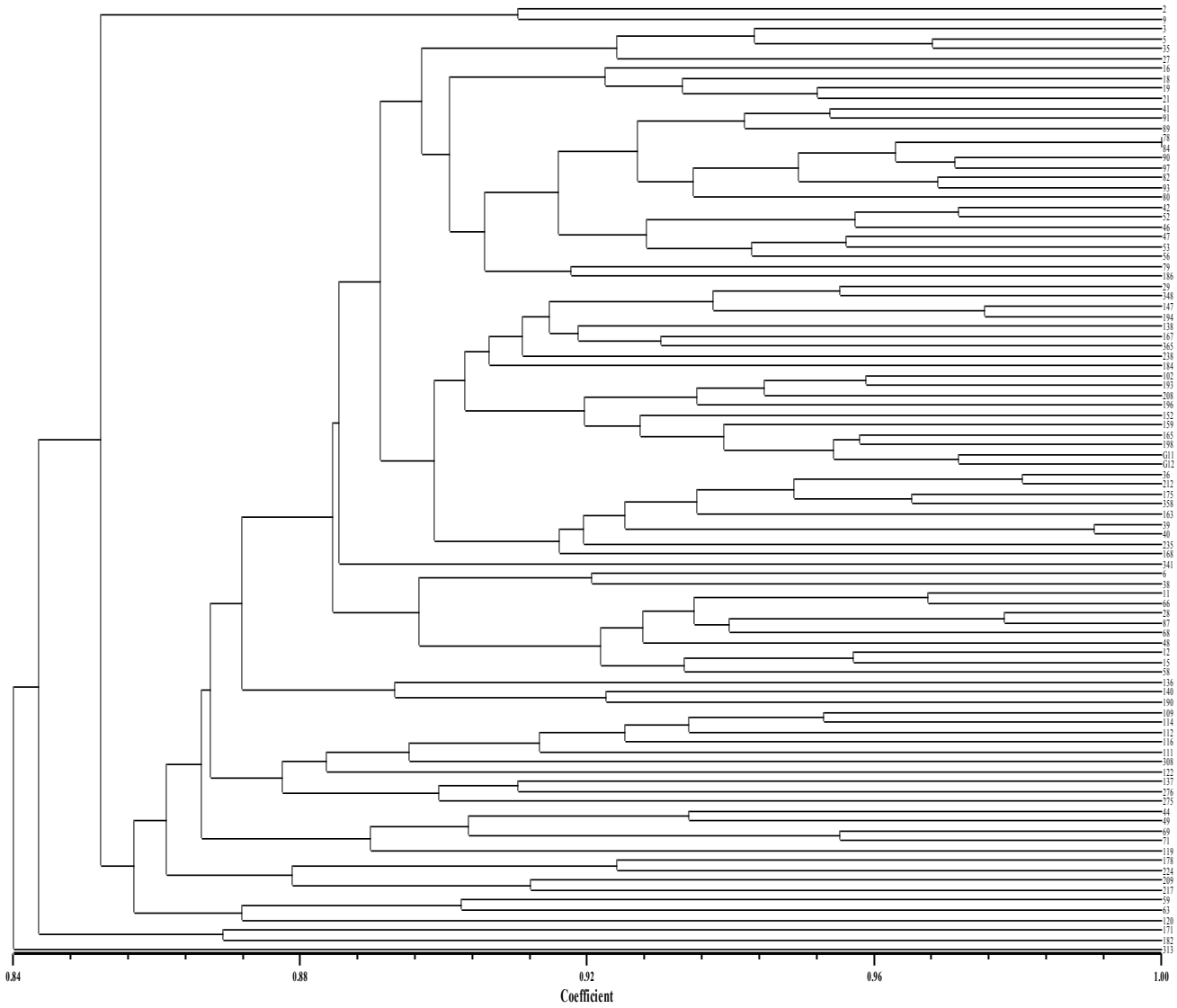


Figure 1. UPGMA dendrogram based on DICE similarity matrix of 96 genotypes

the similarity ratios of genotypes ranged between 0.93-1.00 and these watermelons were extremely close to each other in terms of genetic structure. In a study by Hwang et al. (2011), 6 watermelon varieties were found to be closely related to each other with a similarity ratio of 0.91-0.97. In our study, the similarity rate between watermelons was 0.84-1.00. Although cultivated watermelon genotypes and varieties (*C. lanatus* var. *lanatus*) are highly variable in terms of morphological characters such as skin color and thickness, fruit shape and size, flesh structure and color, sugar content, seed shape and color, it has been reported that the reason why they have limited polymorphism at the DNA level (Navot & Zamir, 1987) may be that the watermelon was cultured outside of its center of origin. As a result of the findings, it was concluded that SSR markers are effective in investigating the genetic diversity of cultured watermelons, which are not very rich in genetic structure. SSR markers have been successfully used to determine genetic diversity among genotypes of different species in watermelons. When the distribution of the markers on the two-dimensional graph is examined, it is observed that some markers are very close to each other, and some are quite far from each other (Figure 2). The closely related markers probably originate from the same chromosome region.

Therefore, the contribution of markers that are close to each other will be lower. This may mean repeating the marker sampling. As a result of this analysis, according to the graph in Figure 2., CMCT505 and CMCT505 were far from each other. According to principal components analysis, the first three eigenvalues were found to be approximately 27. This shows that the first three characters explain only 27% of the total variation. According to the literature, PCA analysis gives meaningful results if the sum of the first three eigenvalues is 25% or more. Therefore, it can be said that PCA results are important in our study.

The distribution of genotypes on the three-dimensional graph was determined by the eigenvector (Figure 3). It was observed that the genotypes 82 to 18, 167 to 147 were close to each other. A distribution compatible with the dendrogram was observed. In a study by Solmaz et al. (2010) genetic diversity in watermelons collected from Turkey was investigated with RAPD markers and the molecular data obtained were evaluated by PCA, and Praecitrullus fistulosus genotypes were grouped separately from other Citrullus

species. It has been determined that the genotypes of the *Citrullus lanatus* species collected from Turkey are densely clustered together (Solmaz et al., 2010). Similarly, in a study by Nantoume et al. (2013), the genetic differentiation of 134 watermelon genotypes was analyzed with 24 SSR primers. As a result, molecular analysis of variance explained 51% of the total variation within populations, while inter-population variation explained 14%

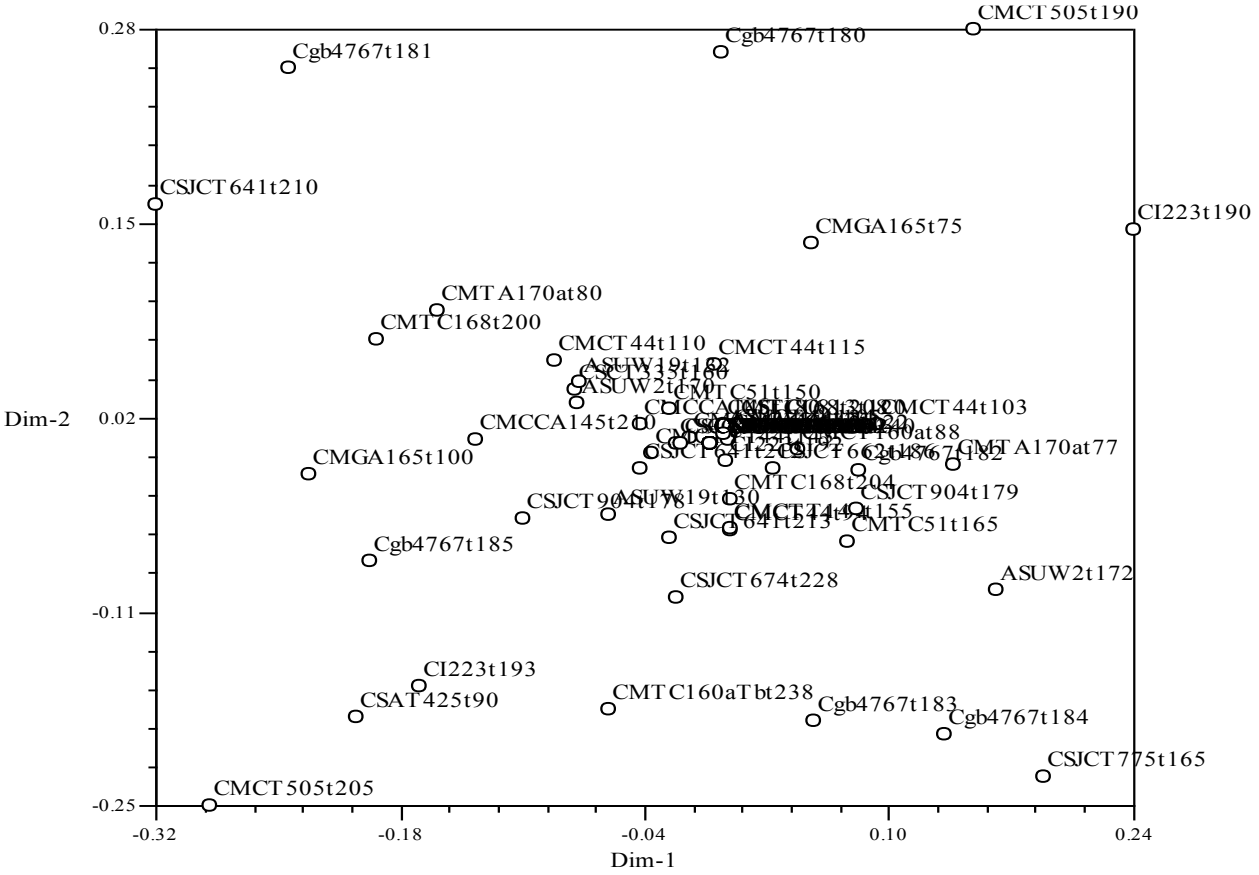


Figure 2. Distribution of markers on a two-dimensional graph

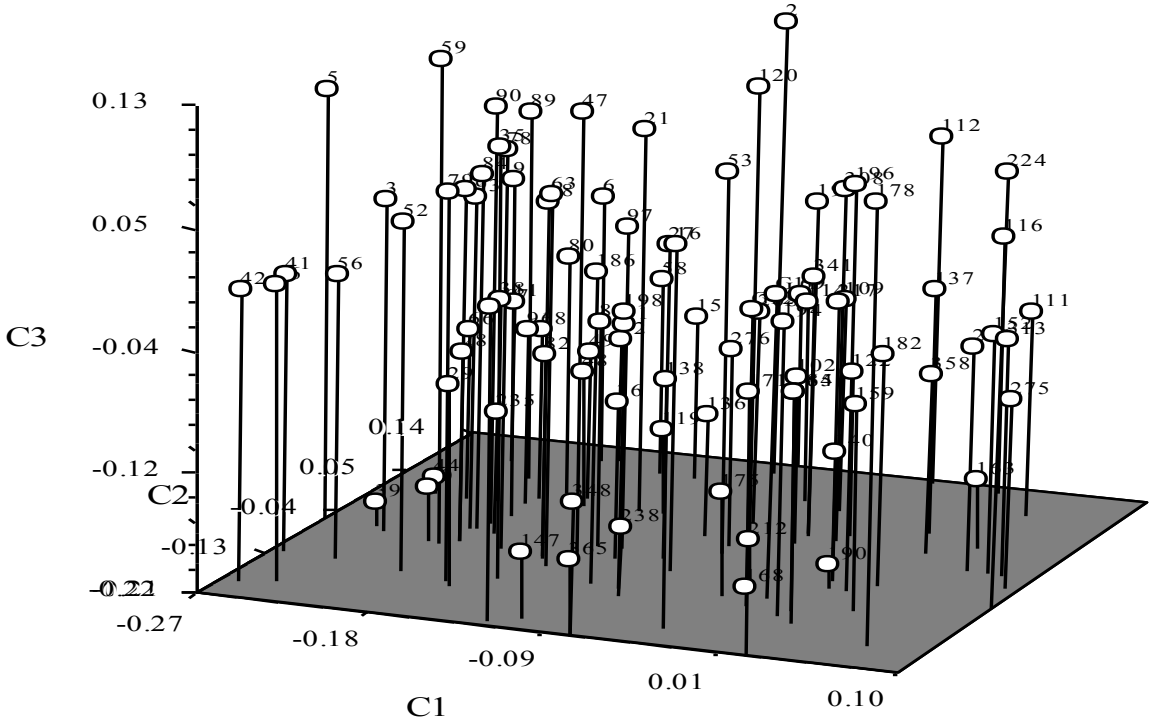


Figure 3. Distribution of genotypes on a three-dimensional graph

## Conclusion

Turkey is a very rich country in terms of plant gene resources due to its geographical location. Although the origin of watermelon is not Turkey, it has valuable varieties in many regions. However, these valuable varieties have come to the point of extinction due to environmental conditions and other pressures. As a result, although they are very different in terms of morphological features, it was determined that these genotypes in the cultured *C. lanatus* species do not genetically have a high level of polymorphism. It is thought that this situation is because Turkey is far from the gene center of watermelon and that wild forms do not grow in our country. Within the scope of this study, very important loci that can be used to determine the purity tests and purification levels among watermelon seeds were determined and presented to the use of breeders.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Not applicable.

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# Assessment of nematicidal activity of *Beauveria bassiana* (Bals.-Criv.) vuill on *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae)

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## Abstract

In this study, nematicidal activity of two different isolates (BY2 and BIM-001) of the *Beauveria bassiana* (Bals.-Criv.) Vuill was investigated on *Pratylenchus thornei* Sher et Allen) (Tylenchida: Pratylenchidae) using culture filtrates and spore suspensions. Three spore suspensions ($1x10^6$, $1x10^7$, $1x\ 10^8$ spore/ml) and four culture filtrate concentrations (1X, 10X, 20X, 50X) were tested in the study. Depending on the treatment; 2 ml of spore suspensions or culture filtrates in different concentrations of both isolates and 400 larvae+adults from *P. thornei* were transferred to 6 mm petri dishes. The dead nematodes were counted under a light microscope after 24 and 72 hour and their mortality rate (%) were calculated. The nematicidal effect of culture filtrates of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* was found to be higher than the spore suspension. It was determined that the 1X concentration of culture filtrate of *B. bassiana* BY2 reached 100% mortality rate on *P. thornei* after 24 hour. After 72 hours, 10X (99.0%) concentrations of BY2 isolate and 1X (100%) and 10X (93.2%) concentrations of BIM isolate showed similar nematicidal activity with the commercial nematicide Velum (97.6%). On the other hand, after 72 hour, *P. thornei* mortality rate was 75.5% and 64.1%, respectively, at a concentration of $10^8$ spore/ml of *B. bassiana* BY2 and BIM-001 isolates. This study will contribute to the development of a new control method as an alternative to the use of crop rotation and resistant cultivars in the control of *P.thornei*.

## Keywords

Culture Filtrate, Nematicidal Effect, Nematicidal Compounds, Entomopathogenic Fungi, Biological Control

## Introduction

Root lesion nematodes (*Pratylenchus* spp.) are migratory plant-parasitic nematodes and cause lesions and necroses in the roots of the plants (Davis et al., 2011). These nematodes lead to significant economic losses that are common in fruits, vegetables and field crops worldwide (Smiley and Nicol, 2009). They damage cells in stems, thus plants cannot properly absorb water and nutrients from the soil, resulting in symptoms similar to nutrient and water deficiency (Castillo and Vovlas, 2007; Kumar et al., 2018). In addition, root lesion nematodes indirectly cause economic losses by helping many soil-borne diseases and pests enter the plant (Smiley et al., 2004; Göze Özdemir, 2020). *Pratylenchus thornei* Sher & Allen 1953 is an economically important pest found in grains and legume roots in many countries of the world (Smiley and Nicol, 2009; Ganguly and Pandey, 2012; Thompson et al., 2017; Mokrini et al., 2019; Kumar et al., 2018). *Pratylenchus thornei* and *P. neglectus* were widely reported in cereal production areas in Turkey (Dababat et al., 2019; Yavuzaslanoğlu et al., 2012, 2020; Göze Özdemir et al., 2021;). It has been reported that cysts and root lesion nematodes in wheat in the Central Anatolian Plateau of Turkey cause about 50% yield loss (Nicol and Ortiz Monastterio, 2004). Field hygiene, use of resistant and tolerant varieties and non-host crop rotation are more preferred for the control of *P. thornei* as in the management of other plant-parasitic nematodes. In addition, cereals cultivation in very large areas makes chemical control less economical. In recent years, biological control of plant parasitic nematodes has been

reported as an important management strategy (Timper, 2014; Li et al., 2015; de Oliveira, 2021). A large number of microorganisms are known as antagonists of plant parasitic nematodes (Akhtar and Malik, 2000). Some soil-borne pathogens have promising potential in the control of plant parasitic nematodes (Anke and Sterner, 1997). Culture filtrates of many entomopathogenic fungi are known to be effective against plant parasitic nematodes, and this nematicidal effect is thought to be caused from toxic metabolites produced by the fungus (Caroppo et al., 1990; Liu et al., 2008; Göze Özdemir and Arıcı, 2021).

*Beauveria bassiana* (Bals.-Criv.) Vuill belonging to entomopathogenic fungi (Hypocreales: Cordycipitaceae) has great potential in the control of many insects and arthropods (Feng et al., 2004; Pu et al., 2005; Hatting et al., 2012; Demirozer et al., 2016; Canassa et al., 2019; Uzun et al., 2021). It has been known that many species of Beauveria secrete secondary metabolites such as bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasse et al., 2000; Quesada and Vey, 2004). These secreted toxins act as a thrombocyte inhibitor in the insect body (Butt et al., 2001). The culture filtrate of *B. bassiana* isolate has been determined to have high nematicidal effect on *Ditylenchus destructor*, *Meloidogyne incognita* (J2), *M. hapla* (J2), *Heterodera glycines* (J2), *Aphelenchoides besseyi* and *Caenarhabditis* sp. (Liu et al., 2007; 2008; Zhao et al., 2013).

There are very few studies about the nematicidal effect of *B. bassiana* on root lesion nematodes. Therefore, this study aimed to determine the nematicidal activity of different spore and culture filtrate concentrations of two different *B. bassiana* isolates (BY2 and BIM-001) originated from Turkey against the root-lesion nematode *P. thornei* under *in vitro* conditions.

## Materials and Methods

### Materials

*Beauveria bassiana* BIM-001 isolate obtained from *Leptinotarsa decemlineata* Say in Isparta potato fields and BY2 isolate obtained from Haplothrips sp. in Burdur wheat fields were used in the study (Sarı, 2020; Uzun, 2020;). Different spore ($10^6$, $10^7$, $10^8$ spore/ml) and culture filtrate concentrations (1X, 10X, 20X and 50X) of these isolates were used. Entomopathogenic fungal isolates were kept in the Integrated Control Laboratory of the Plant Protection Department in Isparta University of Applied Sciences (ISUBU) and renewed periodically to prevent the loss of pathogenicity.

The *Pratylenchus thornei* isolate (SK11) was obtained from a wheat field in Sarkikaraağaç in Isparta, Turkey and identified in previous studies (Göze Özdemir, 2020; 2021). Mass production of *P. thornei* was carried out in carrot discs and renewed every four months in the Nematology Laboratory of the Plant Protection Department in ISUBU (Zuckerman 1985). Nostalgist (*B. bassiana* strain Bb-1, Agrobest Grup Ltd. Şti, Turkey) and Velum (Fluopyram, Bayer Group Co. Ltd.) commercial preparations were used in positive control applications. The distilled water was used as negative control.

### Preparation of the spore suspension

*Beauveria bassiana* BIM-001 and BY2 isolates were cultured on potato dextrose agar (PDA) and incubated for 10 days at 25˚C under dark conditions. Spore suspensions ($10^6$, $10^7$, $10^8$ spore/ml) were prepared by counting spores in a hemocytometer.

### Preparation of the culture filtrate

*Beauveria bassiana* BIM-001 and BY2 isolates were cultured in 250 ml flasks containing 50 ml PDB (potato dextrose broth agar) and sterilized at 121 °C for 20 min. Then, 1 $cm^2$ pieces of 2-week-old fungus colony grown in Potato Dextrose Agar (PDA) were taken into each erlenmeyer flask and incubated at 25 ° C in the dark for 10 days. The flasks were manually shaken every day during the incubation period. After ten days, the culture filtrate was first passed through two layers of filter paper (Whatman No. 1) to remove fungus spores and micelles, then re-filtered through a 0.45 1m pore size filter. Obtained filtrate were used as the pure (1X) culture solution. Other concentrations (10X, 20X and 50X) were prepared by diluting the pure culture with sterile distilled water (Liu et al., 2008). They were kept in the refrigerator at 4° C until used in the experiment.

### Nematode inoculum

The carrot discs in which *Pratylenchus thornei* was produced were transferred to 120 mm petri dishes. Then they were cut into small pieces, and sterile distilled water was added to cover the petri dish. After six hours, the nematodes were extracted using the Baermann funnel method (Mudiope et al., 2004). Adult and larvae of *P. thornei* population were counted under the light microscope at 10X magnification. Then, *P. thornei* nematode density to be used in the study was adjusted and the inoculum was kept in the refrigerator at 4° C in eppendorf tubes containing pure water.

### Nematicidal effect bioassays

The treatments carried out to determine the nematicidal effects of spore and culture filtrates at different concentrations of two *B. bassiana* isolates on *P. thornei* were carried out in plastic petri dishes (6 cm diameter). All treatments were conducted according to a randomized plot design with five replications. The petri dishes were kept at 25 ± 1°C and 60% relative humidity during the treatments. Depending on the treatment group; 2 ml of spore suspensions or culture filtrates of different concentrations of both isolates and 50 microliters (400 larva+adults) from *P. thornei* inoculum were transferred to petri dishes (Liu et al. 2008; Kepenekçi et al. 2018). As a positive control treatment, the nematicidal effects of commercial plant protection products Nostalgist and Velum (at maximum field application doses of 250 ml/da and 120 ml/da, respectively) on *P. thornei* were investigated with the same method. In the negative control, sterile distilled water was used. After 24 and 72 h, dead and living *P. thornei* individuals were counted under the light microscope at 20X magnification and the results were recorded. During counting, the nematode individual that did not move when touched with a thin needle (38X0.30 mm) was considered dead (Cayrol et al., 1989; Zhao et al., 2013). Mortality rates were calculated from the obtained values (Liu et al., 2008).

### Statistical analysis

All data obtained in the study were statistically analyzed by the SPSS 20.0 program. Analysis of

variance (ANOVA) was performed to test the differences between the means of mortality rates. Means of mortality rates at different observation times were compared with Tukey's HSD test at a significance level of $p \leq 0.05$.

## Results and Discussion

### Nematicidal effect bioassays of spore concentrations

The mortality rates at $10^6$, $10^7$ and $10^8$ spore/ml concentrations of BY2 isolate of *B. bassiana* were 9.5%, 20.9% and 42.8% respectively after 24 h whereas these rates in BIM-001 isolate were 5.9%, 16.7% and 35.1%, respectively. It was found that the mortality rates were found higher even at the lowest concentration of *B. bassiana* isolates than the control at 24 h after the application. At the highest concentration of both isolates, the mortality rates at 24 h were found to be below 50%. There were significant differences between the mortality rates for each application dose of *B. bassiana* isolates compared to the control after 24 h. The mortality rates of Nostalgist (*B. bassiana* strain Bb-1), commercial bioinsecticide, were 22.8% and 27.9% at 24 and 72 h, respectively. After 72 h, the nematicidal effect of Nostalgist (22.8%) was higher than *B. bassiana* $10^6$ spores/ml concentration when compared to BIM-001 (15.2%) and BY2 (20.0%) isolates, but lower than $10^7$ and $10^8$ spore/ml concentrations of both isolates. The mortality rates of BIM-001 isolate at $10^7$ and $10^8$ concentrations were 35.9% and 64.1%, respectively while those of BY2 isolate at the same concentrations were 41.7% and 75.5%, respectively after 72 h. The nematicidal effect of *B. bassiana* BY2 isolate was determined to be higher than BIM-001 and a statistically significant difference was found between each spore suspension concentrations of BIM-001 and BY2 isolates ($p \leq 0.05$). It was observed that the mortality rate of *P. thornei* increased directly proportional to the spore concentration in both isolates of *B. bassiana* at 24 and 72 h. The mortality rates of the commercial nematicide Velum (Fluopyram) were 92.2% and 97.6% at 24 and 72 h, respectively. The mortality rates of both isolates at a concentration of $10^8$ spore/ml were over 50% at 72 h after application. A statistically significant difference was found between BIM-001 (64.1%) and BY2 (75.5%) isolates of *B. bassiana* at $10^8$ spore/ml concentration and Velum (97.6%) in terms of mortality rate ($p \leq 0.05$). It was determined that the nematicidal effect of *B. bassiana* differs according to the isolates, spore concentrations of the entomopathogenic fungus and the time passed after the application (Table 1).

### Nematicidal effect bioassays of culture filtrate

The mortality rates of 1X, 10X, 20X and 50X concentrations of *B. bassiana* culture filtrate were 100.0%, 46.5%, 23.1% and 14.7% respectively in BY2 isolate after 24 h, whereas these rates were 85.3%, 48.1%, 20.3% and 10.9% in BIM-001 isolate. It was determined that the mortality rate decreased with the dilution of the culture filtrate. The pure culture filtrate concentration of BY2 isolate (1X) killed all *P. thornei* adult+larvae stages in 24 h. In BIM-001 isolate, the mortality rate was 85.3% at 1X concentration in 24 h, while it was 100% after 72h. It was observed that the nematicidal effect increased over time at other culture filtrate concentrations. The mortality rates of *B. bassiana* BY2 culture filtrate in 1X, 10X, 20X and 50X concentrations were 100.0%, 99.0%, 46.4% and 27.8%, respectively, whereas these rates were 100.0%, 93.2%, 36.5% and 22.1% in BIM-001 isolate. The mortality rates were found to be below 50% at 20X and 50X culture filtrate concentrations of both isolates. However, the nematicidal effect of *B. bassiana* BY2 was higher considering the low culture filtrate concentrations. The difference between 1X concentration of BY2 isolate (100%) and Velum (92.2%) was statistically significant at 24 h, therefore the nematicidal effect of BY2 was higher than the commercial nematicide Velum ($p \leq 0.05$). On the other hand, this difference was not statistically significant for 1X concentration of BIM-001 isolate ($p \geq 0.05$). After 72 h, the nematicidal effect of 10X concentration of BY2 isolate was similar to that of 1X concentration. Also, there was no statistically significant difference between the mortality rates at 1X and 10X concentrations of BIM and BY2 isolates ($p \geq 0.05$). The nematicidal effect of Nostalgist was similar to the effect of 50X culture filtrate concentration of *B. bassiana* BIM-001 and BY2 at 72 h. However, the nematicidal effects of 1X, 10X and 20X concentrations of the culture filtrate in both isolates were higher than Nostalgist at 72 h. The mortality rates of Nostalgist and 50X concentration of the two isolates were significantly higher than the control (the pure water) (Table 2).

It was determined that the nematicidal effect of Turkish isolates named BY2 and BIM-001 of *B. bassiana* obtained from different insects reached 75.5% and 64.1% mortality, respectively, at 72 h in vitro. The mortality rate occurred at $10^8$ spores/ml concentration of *B. bassiana* on *P. thornei* was higher than Nostalgist which was a commercial bioinsecticide containing *B. bassiana*. There are very few studies about the nematicidal effect of *B. bassiana* on root lesion nematodes. Kepenekci et al. (2017) investigated the effect of $10^6$, $10^7$ and $10^8$ cfu/ml-1 suspensions of two Turkish *B. bassiana* isolates (F-56 and F-63) in the tomato field infested with *Meloidogyne incognita* and *M. javanica,* then they reported that $10^8$ cfu/ml-1 suspension in each two isolates showed a nematicidal effect and positively affected the yield.

The nematicidal effect of the culture filtrates of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* was higher than the spore suspensions of the two isolates. The culture filtrates of BIM-001 and BY2 had a high nematicidal effect (1X, 100%) on *P. thornei* at 72 h *in vitro*. Since the culture filtrate is a fermented product, it is thought that the nematicidal effect may have increased with the synergistic or antagonistic effect of the secondary compounds in its content and the possibility of higher amount of enzymes and toxins (Ciancio, 1995; Nitao et al., 1999; Lopez-Llorca et al., 2006; Kim et al., 2013). Extracellular hydrolytic protease and chitinase enzymes produced for cuticle penetration and digestion by fungi used as biocontrol agents are effective on nematodes (Morton et al., 2004; Huang et al., 2004). Regaieg et al. (2010) reported that *M. incognita* eggs exposed to *Verticillium leptobactrum* culture filtrates deteriorated due to the defect in the chitin layers and appeared collapsed. Chitinase and proteinase enzymes purified from *V. suchlasporium* were found to break

down the shells of *Globodera pallida* eggs (Tikhnov et al., 2002).

In the present study, the mortality rates (%) of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* were found to be 100% and 85.3%, respectively, at 1X culture filtrate concentration after 24 h and the nematicidal effect of BY2 isolate was higher than Velum (92.2%). In terms of the nematicidal effect, it was found that Velum (97.6%), 10X (99.0%) concentration of BY2 isolate and 1X (100%) and 10X (93.2%) concentrations of BIM isolate were in the same statistical group after 72 h. Lu et al. (2016) found that the culture filtrate of four out of 10 *B. bassiana* isolates had high virulence against *Pratylenchus* sp. and mortality rates of the BD-B173, BD-B180, BD-B061-3 and BD-B315 isolates were 97.20%, 96.50%, 91.16% and 90.32%, respectively after 24 h. Zhao et al. (2013) determined that the culture filtrate of nine *B. bassiana* isolates completely killed the *Caenarhabditis* sp. within 48 h while caused 15-96% mortality on *M. incognita*, 15-65% on *Aphelenchoides besseyi*, and 51-100% on *Heterodera glycines*. Liu et al. (2008) stated that 1X (96.5%) and 5X (88.9%) concentrations of *B. bassiana* culture filtrate showed high antagonistic activity against J2 of *M. hapla*, and 1X concentration achieved the same mortality as Aldicarb (96.5%). Chen at al. (1996) reported that although the parasitization rate of *H. glycines* eggs of *B.bassiana* was low, larvae hatching was suppressed in parasitized eggs. Sun et al. (2006) found that the parasitization rate of eggs and females of *B. bassiana* was 100% on *Meloidogyne* spp. It was observed in previous studies that the nematicidal effect of *B. bassiana* may differ according to the nematode species.

Table 1. Mortality rates (%) of spore suspension concentrations of different *Beauveria bassiana* isolates on *Pratylenchus thornei*

| | Observation Times* | | | |
|---|---|---|---|---|
| | 24 h | | 72 h | |
| | Entomopathogenic Fungus isolates | | Entomopathogenic Fungus isolates | |
| | BY2 | BIM-001 | BY2 | BIM-001 |
| Treatments | Mean of Mortality rate%± Standart error | | | |
| $10^6$ spore/ml | 9.5±0.3 d A | 5.9±0.6 e B | 20.0±0.9 e A | 15.2±0.8 e B |
| $10^7$ spore/ml | 20.9±0.7 c A | 16.7±0.7 d B | 41.7±1.3 c A | 35.9±1.7 c B |
| $10^8$ spore/ml | 42.8±1.1 b A | 35.1±1.1 b B | 75.5±1.3 b A | 64.1±2.1 b B |
| Nostalgist®(Positive control) | 22.8±0.8 c | 22.8±0.8 c | 27.8±1.1 d | 27.8±1.1 d |
| Velum® (Positive control) | 92.2±1.0 a | 92.2±1.0 a | 97.6±1.1 a | 97.6±1.1 a |
| Distilled water (Negative control) | 0.9±0.1 e | 0.9±0.1 f | 1.1±0.1 f | 1.1±0.1 f |

*Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means differ significantly ($p \leq 0.05$).

Table 2. Mortality rates (%) of culture filtrate concentrations of different *Beauveria bassiana* isolates on *Pratylenchus thornei*

| Treatment | Observation Times* | | | |
|---|---|---|---|---|
| | 24 h | | 72 h | |
| | Entomopathogenic Fungus isolates | | Entomopathogenic Fungus isolates | |
| | BY2 | BIM-001 | BY2 | BIM-001 |
| | Mean of Mortality rate%± Standart error | | | |
| 1X | 100.0±0.0 a A | 85.3±3.7 a B | 100.0±0.0 a A | 100.0±0.0 a A |
| 10X | 46.5±1.4 c A | 48.1±0.8 b A | 99.0±0.7 a A | 93.2±2.8 b A |
| 20X | 23.1±0.9 d A | 20.3±0.7 c B | 46.4±2.0 b A | 36.5±0.8 c B |
| 50X | 14.7±0.5 e A | 10.9±1.1 d B | 27.8±0.9 c A | 22.1±1.4 d B |
| Nostalgist® (Positive control) | 22.8±0.8 d | 22.8±0.8 c | 27.8±1.1 c | 27.8±1.1 d |
| Velum® (Positive control) | 92.2±1.0 b | 92.2±1.0 a | 97.6±1.1 a | 97.6±1.1 ab |
| Distilled water (Negative control) | 0.9±0.1 f | 0.9±0.1 e | 1.1±0.1 d | 1.1±0.1 e |

*Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means differ significantly ($p \leq 0.05$).

## Conclusion

The inoculum method of *B. bassiana* was found important for the nematicidal effect on *P. thornei*, and the culture filtrate method had higher potential than spore suspension. In addition, there was no statistically significant difference between 1X and 10X concentrations of both entomopathogenic fungal isolates (BY2 and BIM-001) and Velum after 72 h under laboratory conditions. These concentrations show similar results with Velum, a chemical nematicide, suggesting that *B. bassiana* BY2 and BIM-001 isolates are promising in the control of *P. thornei* under *in vitro* conditions. It is thought that re-evaluation of the obtained results for culture filtrate concentrations in this

study would be beneficial for application in open field studies for both isolates

## Compliance with Ethical Standards

## Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

## Author contribution

FGGO and AUY participated in the nematicidal effect bioassays and collected of data. OD performed statistically analysis of these data. FGGO, AUY and OD wrote the manuscript, read and approved the final manuscript.

## Ethical approval

Ethics committee approval is not required.

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## Data availability

Not applicable.

## Consent for publication

Not applicable.

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# Determination of the knowledge levels of the farmers of the Diyarbakir region on conservation tillage practices

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## Abstract
This study is performed to determine the knowledge level of farmers in Diyarbakır province about conservation tillage practices to demonstrate the effective methods of producers were aimed. For this purpose, original data collected by a survey from 140 farmers in Diyarbakır province. Existing soil tillage practices of the farmers and the knowledge about sustainable agriculture, green manure, cover crops were defined. It is determined that 57% of the managements tilled the soil by using moldboard plough and 41% of the farmers burns the residue. 72% of the producer send samples for soil analyze but only 87% of them uses soil analyze result in production. 87% of the farmers stated that they have no knowledge and have not heard about conservation tillage likewise 58% of them are about direct seeding.

## Keywords
Survey, Conservation tillage, Direct seeding, Knowledge level

## Introduction
The active tillage adventure of humankind gained speed with the invention of the first wooden plow called “Ard” in the fertile Mesopotamian lands in BC. 4000-6000 years. The plowshares used in Europe in the AD. 5$^{th}$ century, took the most similar shape in AD. 8$^{th}$-10$^{th}$ century to today's plows by gaining the feature of overturning the soil (Anonymous, 2007). Plows in different designs have been used in different geographies, according to regional climate and soil conditions, within the time period that will be expressed in centuries from those years until today. In the 1830s, sales of plows made of cast iron were started by John Deere in the USA. Over time, with the spread of the tractor, which is one of the most important power sources of agricultural production, the use of plows has become widespread. Thus, the plow has become the indispensable main equipment of tillage applications in the world.

Today, the protection of limited or even decreasing land and water resources has gained importance in response to the increasing world population. At this point, the concept of "conservation tillage" has developed. Conservation tillage is a tillage system in which at least 30% of the soil surface is covered with residues or 1120 kg/ha organic matter in order to reduce water and soil erosion and maintain soil fertility (Six et al., 2000; Zhang et al., 2007; Derpsch et al., 2010; Jayaraman et al., 2021). The three key principles of conservation agriculture systems are: (Derpsch et al., 2014):

1- minimizing soil disturbance, consistent with sustainable production practices,

2- maximizing soil surface cover by managing crops, pastures, and crop residues,

3- stimulating biological activity through crop rotations, cover crops, and integrated nutrient and pest management

One of the first attempts towards conservation tillage was the 'soil conservation movement' and 'conservation agriculture', which was initiated in the USA in 1937, as a state policy. In the world, practices related to soil conservation began to develop in the second half of the 20th century (Morgan, 2005). Conservation tillage areas in the world have reached 180 million hectares in 2015/16 period (FAO, 2021). This figure constitutes 14.7% of the total arable land.

When Table 1 is examined, it is seen that USA, Brazil and Argentina take the first three places. As of 2018, the presence of agricultural land in Turkey with conservation tillage is stated as 45000 ha. (FAO, 2021).

Table 1. Amount of agricultural land with conservation tillage by country (FAO, 2021)

| Country | 1000 ha |
|---|---|
| USA | 43 204 |
| Brazil | 32 000 |
| Argentina | 31 028 |
| Australia | 22 299 |
| Canada | 19 936 |
| China | 9 000 |
| Rusia | 5 000 |
| Paraguay | 3 000 |
| Kazakhistan | 2 500 |
| India | 1 500 |
| Uruguay | 1 260 |
| Turkey | 45 |
| Other | 9 666.64 |
| Total | 180 438.64 |

Direct seeding is another planting method that has become widespread around the world in recent years. After the World War II, with the discovery of 2,4-D systemic herbicide and the spread of paraquat herbicide, which started to be sold in 1962, direct seeding method is applied in an increasing amount of agricultural land every year. Direct seeding is most simply defined as the seeding process performed with specially designed direct seeding machines, unlike normal seeding machines, without any tillage after the previous crop harvest (Baker et al., 2007). The success of this process depends on various factors such as suitable crop rotation, effective pest and weed control, selection of suitable machinery-equipment for the system, and the level of knowledge and experience of the implementers. According to 2019 data, direct seeding method is applied in approximately 203 million hectares of land in the world (Anonymous, 2021). The countries that adopt the direct seeding method rapidly are Latin American countries that apply this method on 70% of their arable land. The reason for this rapid increase is that many state-supported institutions, organizations and cooperatives in Latin American countries provided training, practice and financial support to the direct seeding method in the 90s.

When we look at Turkey in general, it is seen that farmers are still far away from conservation tillage and direct seeding practices. Many of the farmers are unaware of these methods, and those who are knowledgeable are hesitant to apply them (Çakır and Aygün, 2016; Altıkat et al., 2018). Studies in this area have not gone beyond the efforts of Universities and Research Centres under the Ministry of Agriculture and Forestry. These studies could not reach the farmers at the desired level. Although exemplary practices have started to be made with the producers in recent years, it is estimated that direct seeding agricultural areas are only 1% of the total arable land in Turkey. (Çelik, 2015). In the survey Studies carried out in different periods and in different regions in Turkey, it has been revealed that the farmers have very little knowledge about coonservation tillage and direct seeding, and those who have knowledge are reluctant to implement it (Küçükkongar et al., 2014; Çay et al., 2015; Turgut ve Barutçu, 2016)

Diyarbakır is a province located on the fertile lands of the Tigris Valley, with a production area of approximately 555000 hectares as of 2020 (TÜİK, 2021). The favorable climatic conditions in the region and the increase in the presence of irrigable agricultural land within the scope of the GAP allowed to grow two crops per year. In parallel with this increase in production, unconscious and intensive tillage has also increased. Also, especially burning the stubble left in the field after the harvest of the second crop, is the most

frequently used method (Sessiz et al., 2010; Öztürk, 2019).

In this study, the current soil cultivation habits of the farmers in Diyarbakır province and its districts, their knowledge levels about conservation tillage and direct seeding methods and their perspectives on these methods were investigated. In addition, it has been tried to learn under which conditions farmers can abandon conventional tillage methods, especially stubble burning.

## Materials and Methods

The material of the research consisted of original data collected by questionnaire from agricultural business owners who carry out plant production in Diyarbakır province and its districts. The districts, villages and towns to be surveyed were selected from the I. Sub-Region, which has the best agricultural conditions according to the soil structure of the lands where crop production is intense, the land capability classes of the agricultural lands and the cultivation period. Sur, Kayapınar, Bismil, Ergani, Silvan and Çınar districts within this region were randomly selected and the farmers were interviewed face-to-face by going to the relevant region. A total of 140 farmers representing agricultural enterprises were surveyed. The sample number was determined with 95% confidence interval, 10% margin of error and 50% response rate using simple random sampling method.

## Results and Discussion

The total land assets of the farmers participating in the survey is 3416.5 ha. Production is carried out in irrigated conditions in 772.0 ha of this land and in dry conditions in 2644.5 ha. When we look at the crop pattern, most of the farmers in dry farming conditions cultivating wheat+lentil or wheat+barley as the main crop. Farmers who cultivate in irrigated conditions produce wheat+cotton or wheat+corn. The distribution of land sizes belonging to the enterprises is shown in Table 2.

Table 2. Land size distribution of enterprises

| Cultivated area (da) | Distribution (%) |
|---|---|
| <50 | 13 |
| 51-100 | 19 |
| 101-150 | 14 |
| 151-200 | 10 |
| 201-250 | 7 |
| 251-300 | 8 |
| 301-350 | 9 |
| 351-400 | 2 |
| 401-450 | 1 |
| 451-500 | 5 |
| 501-1000 | 11 |
| >1000 | 1 |
| TOTAL | 100 |

As it can be seen in Table 2, enterprises with sizes between 51-100 decares and 101-150 decares are higher in terms of land assets among the enterprises included in the survey. When the education levels of the farmers participating in the survey were examined, it was determined that 46% of them were primary school graduates, 18% were secondary school graduates, 23% were high school graduates and 13% were university graduates. (Figure 1).

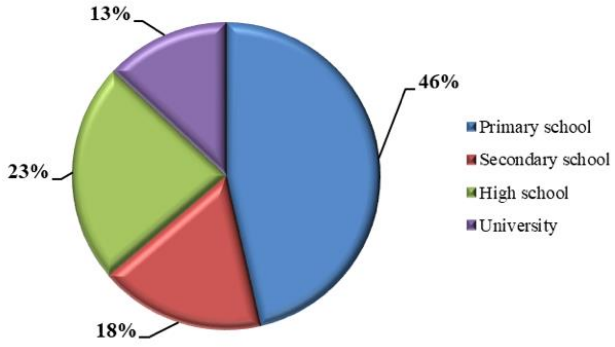


Figure 1. Education level of the farmers participating in the survey

It has been determined that 87% of the enterprises are registered to the National Record of Farmers (NRF). 72% of farmers have soil analysis. 87% of the farmers who have soil analysis take soil analysis results into consideration in production.

It has been observed that farmers obtain their equipments and machines by purchasing 63%, by leasing 25% and by borrowing 12%. (Figure 2).

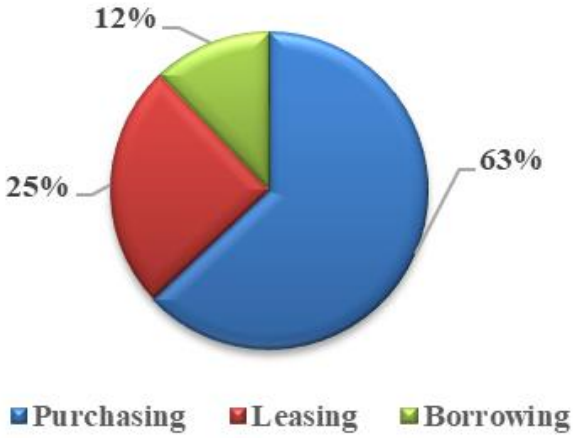


Figure 2. Ways of enterprises to supply equipments and machines

When the data on the tillage habits of the enterprises within the scope of the survey were examined, it was seen that the conventional tillage method, in which the plow was used in the field preparation before planting, was used with a rate of 57% throughout the province. It was determined that the farmers had soil analysis with a rate of 72% and the majority of them (87%) complied with this analysis in fertilization planning. Küçükkaya ve Özçelik (2014), in their survey conducted with the farmers in the Gölbaşı district of Ankara, it was determined that the farmers who did not comply with the results of the soil analysis were more than those who comply with the results of the analysis with a rate of 56.66%.

Table 3. Pre-seeding practices of enterprises

| | Yes | No |
|---|---|---|
| Cultivating second crop | 83% | 17% |
| Stubble burning | 41% | 59% |
| Had soil analysis | 72% | 28% |
| Considers soil analysis | 87% | 13% |

Continuous stubble burning is a widespread application in Turkey (Akbolat et al., 2016; Altıkat et al., 2018; Çelik et al., 2019). This practice still continues throughout the region and the province. 57% of the farmers in 41% group of farmers who burn stubble, know the harms of burning stubble, but still prefer to burn stubble. When the farmers within the scope of the survey were asked in which case they would give up burning stubble, the majority of them answered if the straw became valuable (52%) and if financial support was given to the direct seeding. On the other hand, the rate of producers who stated that they would give up burning stubble if the penalties for burning stubble were increased remained at only 7%. This situation, which emerged with the survey, showed that the reward and support system would be more effective than the punishment method at the point of adopting new methods. Figure 3 shows in which situations the farmers can give up burning stubble.

57% of the farmers stated that they use plows before each planting period. It was observed that the level of knowledge of the farmers about the relationship between tillage and soil organic matter (SOM) and soil erosion was low. (Table 4). 88% of the 43% farmer group who know both of these relationships continue to use plows.

In the Council of Ministers Decision dated 29.12.2015 and numbered 2015/8353 published in the Official Gazette dated 01.01.2016 and numbered 29580; VAT rates, SCT rates and amounts and tobacco fund amounts to be applied to some goods have been redefined (Anonim, 2016). Among these goods are fertilizers registered by the Ministry of Agriculture and Forestry. In the following years, there was an increase in fertilizer prices again due to the exchange rate difference. This has affected the purchasing power of farmers and the amount of fertilizer use has decreased throughout the country. In the survey, farmers were asked whether there would be a change in the amount of fertilizer they use in case of a decrease in fertilizer prices again. 57% of the enterprises stated that they would use more fertilizer if fertilizer prices decreased.

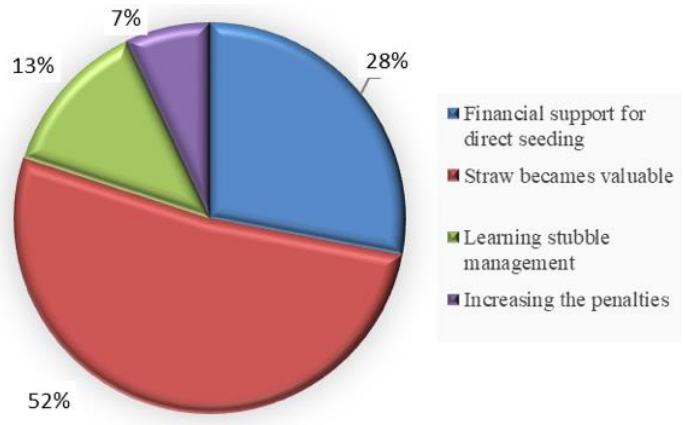


Figure 3. Conditions for farmers to stop burning stubble

Table 4. Pre-planting practices of farmers

| | Yes | No |
|---|---|---|
| Uses plow | 57% | 43% |
| Knows the relationship between tillage and SOM | 48% | 52% |
| Knows the relationship between tillage and erosion | 60% | 40% |
| Knows both | 43% | 57% |

Table 5. Knowledge level of farmers about some conservation tillage terms

| Term | I have not heard and I have no knowledge | I have heard but I don't know | I have heard and I have knowledge | I am applying |
|---|---|---|---|---|
| Sustainable agriculture | 25% | 34% | 41% | 0% |
| Green manuring | 37% | 28% | 35% | 0% |
| Cover crop | 28% | 31% | 41% | 0% |
| Conservation tillage | 51% | 36% | 16% | 0% |
| Direct seeding | 38% | 20% | 42% | 0% |

It has been observed that the level of knowledge and especially the application of sustainable agriculture, green manuring, cover crop and direct seeding practices, which are among the methods included in the concept of conservation tillage, are quite low among the farmers within the scope of the survey (Table 5). Of the surveyed farmers, 51% stated that they had never heard of the term "conservation tillage" and 38% stated that they had never heard of the term “direct seeding” before and had no knowledge of it. From the answers given to the questionnaire, it was determined that while the rate of farmers who heard all of the terms in Table 5 was 4%, there was an 11% farmer group who had not heard of any of these terms before and had no knowledge of them. This farmer group also did not feel the need to access information about these issues from anywhere.

When the sources from which the producers accessed information about conservation tillage and direct seeding were examined, it was determined that the vast majority obtained information from the Provincial/District Directorates of Agriculture and Forestry (42%) and Farmer TV (36%) (Figure 4). Çay et al. (2015) determined that the farmers in the Çanakkale region mostly reach the information about the conservation tillage concepts via Farmer TV and the internet. Gülter et al. (2018) ve Turgut ve Barutçu (2016) similarly, in their studies, determined that the farmers primarily accessed technical information via TV and the internet, and that official institutions and universities remained in the lower ranks in terms of being a source of information.

38% of the farmers who participated in the survey stated that they had never seen a direct seeding machine before. Farmers stated that they saw the direct seeding machine mostly at the fair (52%) (Figure 5).

73% of the 62% of the farmers who have seen the direct seed drill know the difference between the direct seeding machine and the normal seed drill.

The producers within the scope of the survey were asked about their willingness to participate in a training program on conservation tillage and direct seeding methods. 90% of the farmers stated that they would like to participate if the training was organized (Figure 6).

The farmers within the scope of the survey were asked under which conditions they could try conservation tillage and direct seeding methods. 46% of the producers stated that they would make trial plantings if they were given training support, and 43% said that they would be given financial support. The rate of farmers who stated that they could make trial seedings if the method became widespread, along with financial support, remained at 9% (Figure 6).

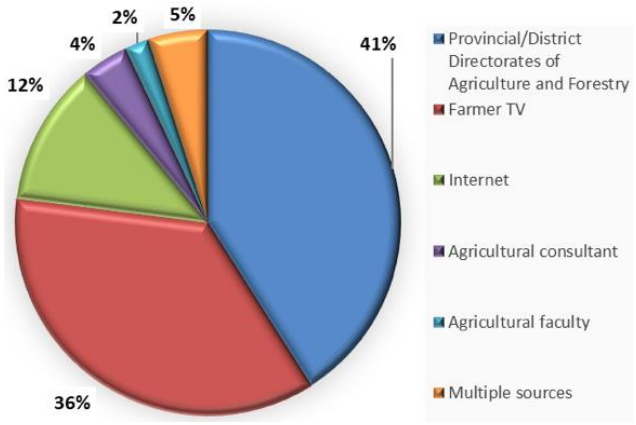


Figure 4. Ways for farmers to learn about conservation tillage terms

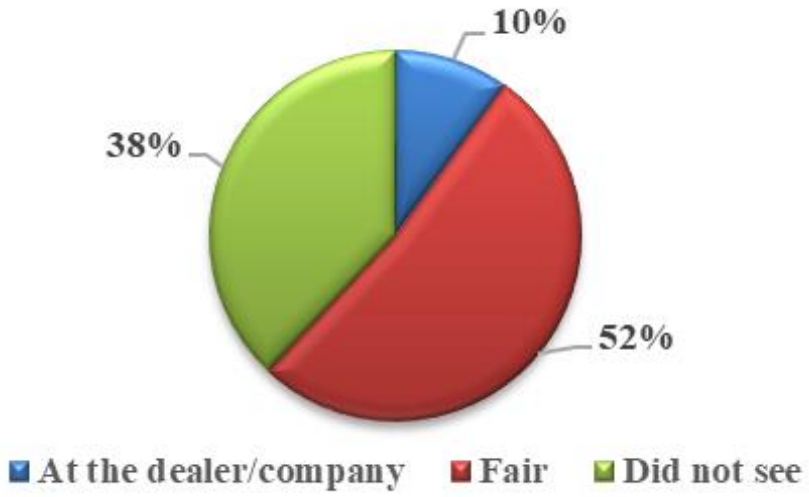


Figure 5. Where the direct seeding machine sees

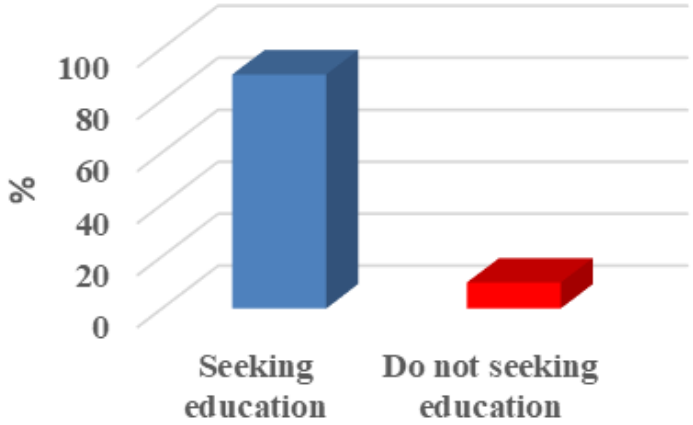


Figure 6. Farmers' willingness to receive training in conservation tillage and direct seeding

Table 6. Conservative tillage and direct seeding trial conditions of farmers

| Conservative tillage and direct seeding trial conditions | % |
|---|---|
| Provided training and application support | 46 |
| Financial support | 43 |
| Financial support-Educational support | 9 |
| If it becomes widespread in the region | 2 |

## Conclusion

When the information obtained within the scope of the research is evaluated, it has been observed that the knowledge level of the farmers about the conservation tillage and direct seeding methods is quite low in the I. Sub-Region, where agriculture is intensively made in Diyarbakır. Although the farmers are eager to learn the concepts of conservation tillage and direct seeding, they are reluctant to implement them without educational support and financial support. Both the low profit margins in domestic agricultural production and the low competitiveness in foreign markets are one of the most important obstacles for farmers to adopt new production techniques. Because our farmers do not have the luxury of taking financial risks for new methods of which they do not know the end. Dissuading farmers from their centuries-old soil cultivation habits and making them adopt new production methods can only be achieved through socio-economic planning. When we look at the examples in the world, it is seen that governments firstly spread conservation agricultural policies on a national basis, and then provide serious training and financial support to conservation tillage and direct seeding practices as sub-factors. While providing these funds, support is received from many international organizations such as FAO.

Within the scope of the survey, it has been observed that farmers tend to abandon the use of plows over time due to increased fuel costs, moisture loss in the soil, etc. Some farmers are not aware that the methods they apply are within the scope of conservation tillage. It has been seen that the education, reward and incentive system should be introduced in order for the producers to adopt new agricultural practices and especially the harms of stubble burning, and the penalty system is no longer a deterrent to the farmers.

It has been revealed that farmers try to reach information on conservation tillage and direct seeding from farmer TVs and the internet rather than Agriculture Faculties and agriculture provincial/district directorates. Based on this, it is seen that the knowledge and experience of agriculture faculties and agriculture provincial/district directorates are weak in transferring them to farmers. Perhaps, this knowledge and experience should be conveyed through visual, audio and written media, taking into account the new habits of the society.

Establishing a data bank similar to this study, which was carried out in the example of Diyarbakir province, with studies to be carried out in different regions of Turkey, and follow a national strategy according to these data will be beneficial in terms of establishing the concept and practices of conservation soil cultivation and direct cultivation.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Ethics committee approval is not required.

### Funding

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Adsorptive removal of cationic dye from aqueous solutions using Bardakçı clay

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## Abstract

This study was investigation employed the batch adsorption method using Bardakçı Clay/Van as an adsorbent to remove crystal violent, a cationic dye from an aqueous solution. The effects of pH, adsorbent amount, interaction time and concentration were investigated to determine the optimal adsorption conditions. The optimal adsorption conditions were determined to be pH=6, 0.6 g adsorbent amount, and the adsorption reached equilibrium at the 25th minute. The equilibrium isotherm was determined using the Langmuir, Freundlich and Dubinin–Radushkevich (D–R) adsorption equations. It was discovered to conform to the Langmuir isotherm. In the Dubinin-Radushkevich (D-R) model, the E value was calculated to E< 8 kJmol$^{-1}$, indicating that the adsorption process occurs physically. Thermodynamic parameters such as enthalpy ($\Delta H^o$), Gibbs' free energy ($\Delta G^o$) and entropy ($\Delta S^o$) were calculated. The adsorption of crystal violet with Bardakçı clay demonstrated that the process was endothermic, occurring both physically and spontaneously.

## Keywords

Bardakçı Clay, Crystal Violet, Adsorption, Adsorption isotherms, Thermodynamics

## Introduction

All living organisms require water to survive (Atasoy, Mercan, Alacabey, & Kul, 2011). Today, water pollution has become a serious concern to humans due to rapid industrialization and population growth (Bhatnagar, Jain, & Mukul, 2005; Senthilkumaar, Kalaamani, & Subburaam, 2006). However, the scarcity of clean water supplies has developed into a significant problem as a result of pollution caused by the discharge of untreated industrial wastewater. Environmental pollution in developing countries has gotten worse due to the uncontrolled population growth, the use of obsolete practices and technologies that consume large amounts of water in agricultural and industrial operations and requires immediate attention (Lee, Low, & Gan, 1999; Saeed, Sharif, & Iqbal, 2010).

Various dyes are used to colour a wide variety of industrial products, including food, textiles, paper, rubber, plastic, cosmetics, medicine, etc(R. Ahmad, 2009; Banat, Nigam, Singh, & Marchant, 1996; Kumar & Ahmad, 2011). The dye is responsible for many problems and is called stubborn dye due to its non-degradable natüre (W. Ahmad et al., 2021). As a result, a significant amount ofcoloured waste is generated and discharged into water resources (R. Ahmad, 2009; Banat et al., 1996; Kumar & Ahmad, 2011).

Crystal Violet (CV), a purple dye, is a triarylmethane dye that is widely used in the textile industry to dye silk and cotton (Chakraborty, Chowdhury, & Saha, 2011; A. Mittal, Mittal, Malviya, Kaur, & Gupta, 2010; Senthilkumaar et al., 2006), therefore, a minute quantity of CV in water can reduce the access of sunlight and disturb the photosynthesis process(H. Mittal, Al Alili, Morajkar, & Alhassan, 2021). Additionally, it is used to manufacture dye and printing inks (Chakraborty et al., 2011; A. Mittal et al., 2010; Senthilkumaar et al., 2006).

CV is used as a gram stain in medicine, as a bacteriostatic agent in veterinary (A. Mittal et al., 2010) and as an external skin disinfectant in humans (Kumar & Ahmad, 2011). It is also used as an additive to prevent the spread of mould, intestinal parasites, and fungi in poultry feed (Kumar & Ahmad, 2011). CV is carcinogenic, a re-polluting molecule, poorly metabolized by microbes, non biodegradable, and persistent in various environments. CV causes moderate eye irritation and painful light sensitivity. Because it contains cationic dye, it has the potential to cause permanently damage the conjunctiva and cornea. It is highly toxic to mammalian cells and, if absorbed through the skin in harmful amounts, may cause skin and gastrointestinal irritation. In cases of overexposure, it can also lead to respiratory and renal failure (R. Ahmad, 2009; Chakraborty et al., 2011; A. Mittal et al., 2010; Saeed et al., 2010).

Water pollution caused by synthetic dye molecules is detrimental to the environment and has adverse effects on public health (Akar, Özcan, Akar, Özcan, & Kaynak, 2009; Chakraborty et al., 2011; Kiran, Ilhan, Caner, Iscen, & Yildiz, 2009). As a result, the majority of these dyes are toxic, carcinogenic or mutagenic. As a result, coloured wastes must be treated appropriately before being discharged into water sources (R. Ahmad, 2009; Choy, McKay, & Porter, 1999; Inthorn, Singhtho, Thiravetyan, & Khan, 2004; Kumar & Ahmad, 2011).

Commonly used methods for eliminating inorganic and organic wastes that contribute to environmental pollution and harm human health include chemical precipitation, ion exchange, membrane filtration, coagulation and adsorption (Bhattacharyya & Gupta, 2008; Caliskan, Kul, Alkan, Sogut, & Alacabey, 2011; Lin & Juang, 2002; Riza, Tolga, Ihsan, Salih, & Yunus, 2011).

Each of them has a number of advantages and disadvantages. Among these approaches, the adsorption technique is the most popular due to its high efficiency, simplicity of design, and ease of use. The main benefits of a potentially successful alternative adsorption for dye removal in wastewater are its adaptability, ease of design, and the fact that it does not lead to the formation of hazardous substances (Derakhshan & Moradi, 2014; Iriarte-Velasco, Chimeno-Alanís, Gonzalez-Marcos, & Álvarez-Uriarte, 2011; Yao, Xu, Chen, Xu, & Zhu, 2010). Various materials are used as adsorbents to purify water. Commonly used adsorbents include; kaolinite (Riza et al., 2011), montmorillonite (Bhattacharyya & Gupta, 2008), pumice (İhsan Alacabey et al., 2021), sediment (İhsan Alacabey, Kul, Şakir, & Alkan, 2020), chitosan (Acet et al., 2018), active carbon (Depci et al., 2011), zeolite (Ji et al., 2021), diatomite (Kul, Alacabey, & Kılıç, 2010).

The primary objective of this study is to remove CV dyestuff from synthetic waters using an adsorption method with clay (BK adsorbent) taken from Bardakçı village in the province of Van. Thermodynamic studies and adsorption isotherm models were examined to evaluate the experimental data. In addition, the BK adsorbent has been characterized.

## Materials and Methods

### Preparation of Adsorbent

BK adsorbent samples were obtained 5cm below the surface after matching in order to prevent contamination.

The BK adsorbent was ground in a mortar and filtered through a 230 mesh sieve for washing. 100 grams of BK adsorbent were washed in 1.7 L of distilled water while, stirring at 150 rpm for 12 hours. After the mixing time was complete, it was left for 12 hours to allow the aqueous phase to separate from the solid phase. The solid phase was filtered and then left to dry for one week at room temperature. The dried BK adsorbent was transferred to a vessel after being sieved through a 230 mesh sieve. Bardakçı clay was stored in a desiccator until used in the experiment. (İ Alacabey, 2006).

### Chemicals

Crystal Violet ($C_{25}N_3H_3OCl$) dyestuff, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH) used in the study were obtained from Sigma – Aldrich company. These chemicals supplied are of analytical purity.

### Devices

The BK adsorbent's physicochemical properties were determined using standard procedures described by (Riza et al., 2011). The adsorbent was analyzed using a Thermo Scientific ARL Perorm'x Wavelength Distributed X-ray Fluorescence (WD-XRF) Spectrometer to determine the oxides present in the structure. Fourier transform infrared (FTIR, 400 – 4000 $cm^{-1}$ range, Bruker Vertex 70) was used to determine the functional groups present in the BK adsorbent. An X-ray diffraction (XRD, Philips PW 1830-40) device was used to determine the diffraction pattern and interplanetary spacing of the BK adsorbent. The surface morphology of the adsorbent was determined by Scanning Electron Microscopy (SEM, Zeiss). The surface area measurement was performed by Brunauer–Emmett–Teller (BET, Nova 2200e Quantachrome Instruments). The absorption measurements were performed using the UV-Vis spectrophotometer (λmax=592 nm for Pg instruments, T80+). Centrifugation (Nüve Nf 200) was used to precipitate the adsorbent.

### Method

Adsorption studies were carried out using the batch system method.

### Adsorption studies

A dyestuff stock solution of 2000 $mgL^{-1}$ diluted to the required starting concentrations was prepared. The following parameters were examined during dye adsorption: pH (2, 4, 6, 8), amount of adsorbent (0.05 g, 0.1 g, 0.2 g, 0.4 g, 0.6 g, 0.8 g, 1.0 g), concentration (10, 30, 50, 70 and 100 $mgL^{-1}$ ), varying interaction times (0, 1, 3, 5, 7, 10, 15, 25, 35, 45, 60, 75, 90 min), and temperature (298 K, 313 K, 323 K). The adsorption capacity ($mgg^{-1}$) and adsorption percentage (%) were obtained from the following equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

$$\text{Adsorption } (\%) = \frac{C_0 - C_e}{C_0} x100 \quad (2)$$

In Equations 1 and 2, $C_o$: is the initial concentration of the CV. dyestuff in the solution ($mgL^{-1}$); $C_e$: final CV dyestuff concentration in solution ($mgL^{-1}$); V: CV is the volume of the dyestuff solution (L), m: is the weight of the BK adsorbent (g), and $q_e$ ($mgg^{-1}$) is the adsorption

amount at equilibrium (Erol, Yıldız, Alacabey, Karabörk, & Uzun, 2019).

## Results

### Characterization

### XRF Analysis

The quantitative chemical analysis of Bardakçı clay by X-ray fluorescence spectrometry technique is given in table 1.

The findings indicate that the percentage of CaO is quite high. The higher percentage of CaO compared to $Na_2O$ indicates that the sample is predominantly composed of calcium bentonite (Ca-B). The absence of permanent suspensions in water, that is, the prepared suspensions collapse rapidly and do not swell significantly in water, demonstrates that the sample is Ca-B (Biçer, 2019; Yalçın, 2010).

As a result of XRF analysis, a $Na_2O + K_2O/CaO + MgO$ value ≥ 1 in metal oxides is classified as Sodium Bentonite (Na-B), Intermediate Bentonite when $1/3 < Na_2O + K_2O/CaO + MgO$ value < 1, $Na_2O + K_2O/CaO + MgO$ when its value is less than 1/3, it is considered as Calcium Bentonite (Ca-B) (Yalçın, 2010). As shown in Table 1 the $Na_2O + K_2O/CaO + MgO$ value is less than < 1/3. This demonstrates that the adsorbent is a Ca-Bentonite type clay. The results obtained are consistent with the literatüre.

Table1. XRF Analysis of BK adsorbent

| A. Za % | $Al_2O3$ % | CaO % | $Fe_2O3$ % | $K_2O$ % | MgO % | MnO % | $Na_2O$ % | $P_2O_5$ % | $SiO_2$ % | $TiO_2$ % |
|---|---|---|---|---|---|---|---|---|---|---|
| 12.5 | 10.4 | 16.1 | 6.4 | 1.6 | 5.8 | 0.1 | 1.6 | 0.2 | 43.7 | 1.3 |

### XRD Analysis

X-ray powder diffraction analyses of BK adsorbent are shown in Figure 1.

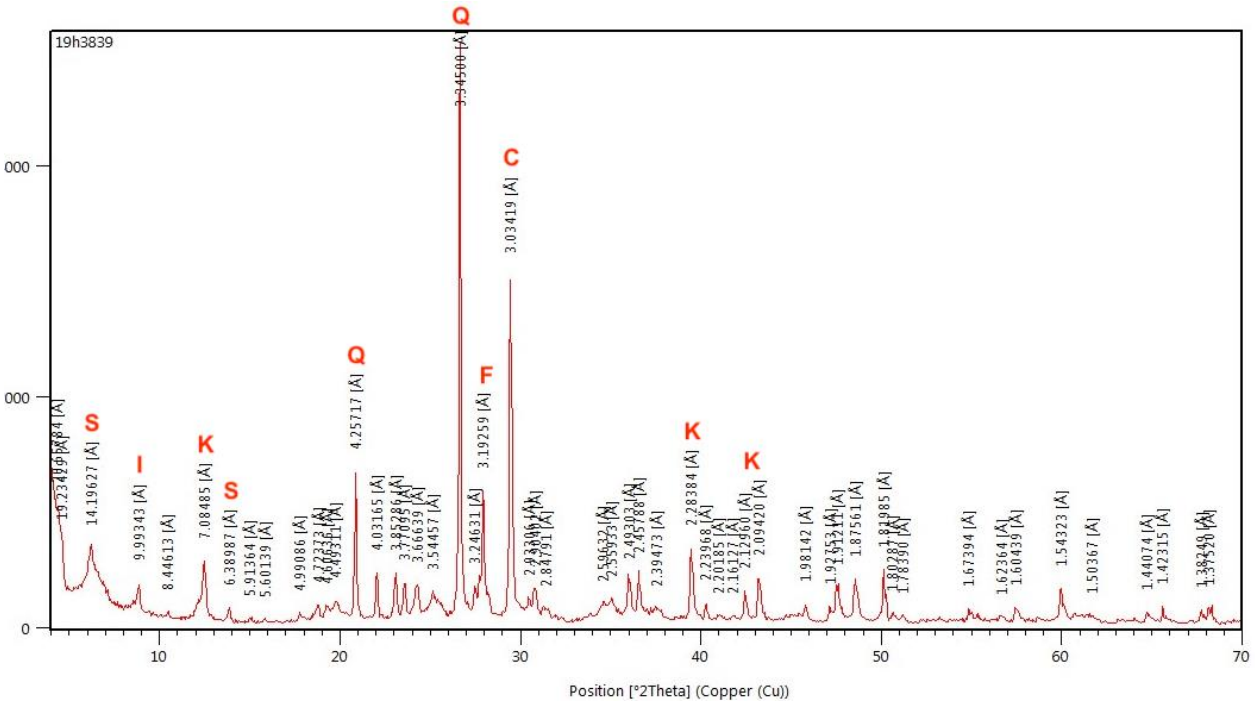


Figure 1. XRD analysis of BK adsorbent (S: Smectite, I: Illite, K: Kaolinite, Q: Quartz, C: Calcite, F: Feldspar)

Qualitative detailed clay analysis, revealed that the BK adsorbent was composed of calcite, quartz, illite, plagioclase, dolomite, chlorite, alkali feldspar and mica minerals.

### FTIR Analysis

The FTIR spectrum of the BK adsorbent is shown in Figure 2.

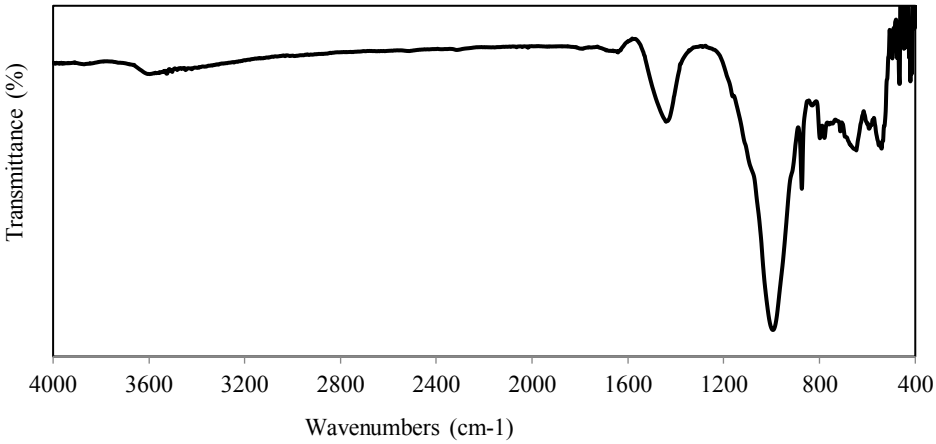


Figure 2. FTIR spectrum of BK adsorbent

The peak in 3604 $cm^{-1}$ is in the –OH groups and is caused by the H–O–H vibrations of the water absorbed on the clay surface (Caliskan, Sogut, Savran, Kul, & Kubilay, 2017; Felhi, Tlili, Gaied, & Montacer, 2008; Madejova & Komadel, 2001). The significant sharp peak at 1435 $cm^{-1}$ indicates Si-O stretching absorption and confirms the presence of a high amount of calcite in the clay. The characteristic bands of the calcite mineral are 1435, 873 and 713 $cm^{-1}$. The 1641 $cm^{-1}$ band corresponds to the vibration of water molecules (H-O-H) trapped in the silica matrix. The IR peak at 1795 $cm^{-1}$ and 2515 $cm^{-1}$ also indicates the presence of calcite and dolomite. The strong band at 993 $cm^{-1}$ (due to Si–O stretching) is the main characteristic band of quartz (Biçer, 2019; Caliskan et al., 2017). Other quartz bands at 798 and 779 $cm^{-1}$ are also present (Caliskan et al., 2017; Gadsden, 1975). The band at 873 $cm^{-1}$ is bound to Fe-Al-OH of montmorillonite (Caliskan et al., 2017; Felhi et al., 2008; Madejova & Komadel, 2001). The bands at 495 and 476 $cm^{-1}$ are attributed to bending vibrations of Si-O-Al and Si-O-Si, respectively. The stretching vibration of adsorbed water molecules is observed at 3419 $cm^{-1}$. Another characteristic band for bending vibrations of adsorbed water appeared as a small band at 1635.64 $cm^{-1}$. Stretching vibrations of surface hydroxyl groups (Si-Si-OH or Al-Al-OH) are observed at 3525 $cm^{-1}$ (Caliskan et al., 2017; Madejová, 2003). The results obtained are consistent with the literature.

### SEM Analysis

The examination of the BK adsorbent by scanning electron microscopy is presented in Figure 3.

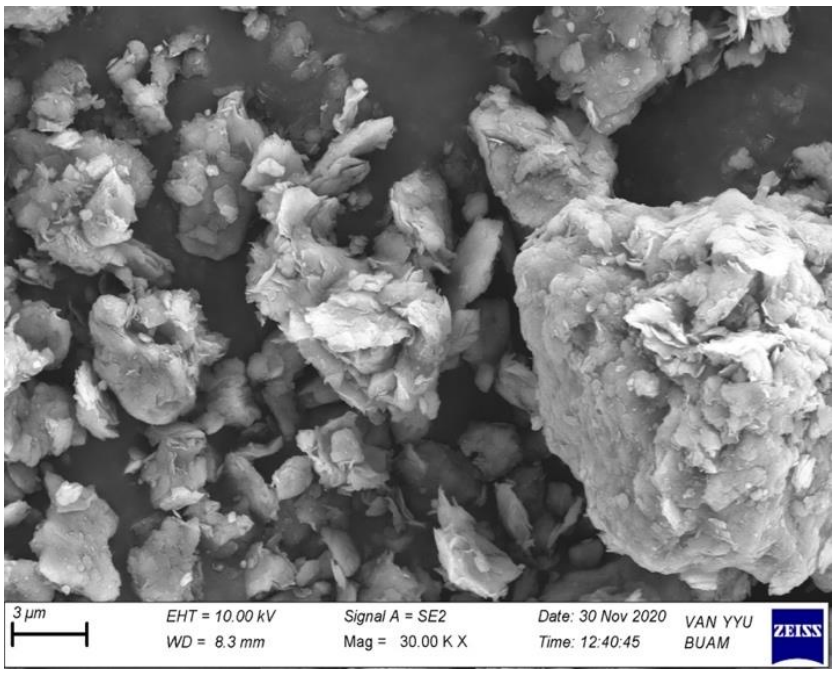


Figure 3. Scanning electron microphotograph of BK adsorbent

The SEM image of the BK adsorbent reveals a variety of sizes and shapes. Quadrangular structures represent calcite, hexagonal structures represent chlorite, hexagonal prism-like shapes represent quartz, shapes like curly lettuce leaf represent smectite (montmorillonite), hexagonal-shaped plates show kaolinite, shapes like elongated branches represent illite (Caliskan et al., 2017; Karakaya, Karakaya, & Faure, 2007).

### BET Analysis

The specific surface area of the BK adsorbent was determined as 57.10 $m^2g^{-1}$.

## Adsorption Studies

### Effect of pH

CV solutions were produced using HCl and NaOH at a concentration of 10 $mgL^{-1}$ at 313 K and pH levels (2, 3, 4, 5, 6, 7, 8). 0.2 g BK adsorbent was used in the batch system to investigate the pH effect. The results obtained are shown in Figure 3.

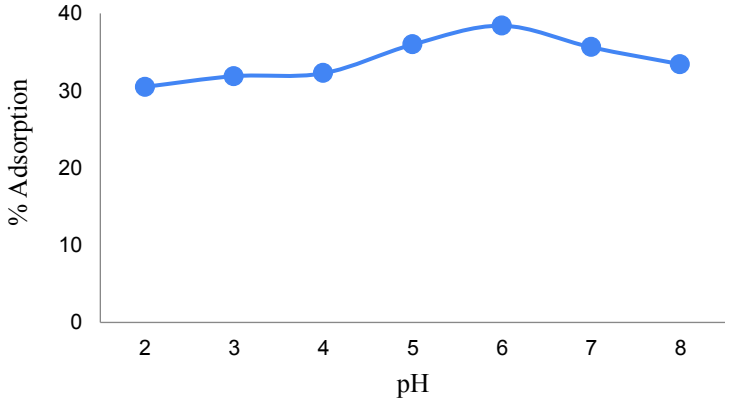


Figure 4. The effect of pH on BK adsorbent of CV dyestuff (adsorbent amount = 0.2 g, concentration =10 $mgL^{-1}$, temperature = 313 K)

The percentage of CV dyestuff adsorption (%) versus the pH values of the BK adsorbent is given in Figure 4. It is understood that the CV dyestuff selectivity of the BK adsorbent increases at pH=6. The optimum adsorption percentage was obtained at pH = 6. The effect of pH on adsorption is related to its ability to ionize adsorbing molecules. The number of positively charged ions on the surface of the BK adsorbent reaches a maximum at pH=6. Due to the cationic nature of CV, the adsorption percentage increases with increasing pH, as the adsorption capacity and the number of OH- ions accumulated on the adsorbent surface increase. The low CV dye removal performance of BK adsorbent in pH >6 and pH <6 solutions is due to the fact that dye cations do not prefer adsorption due to the existing electrostatic repulsion. In addition, the presence of $H^+$ ions competing for adsorption sites on the BK adsorbent with dye cations reduces CV adsorption. The reduced percentage of dye removal in an alkaline medium may be due to adsorbent surface hydrolysis, which creates positively charged areas (Collins & Elijah, 2019; Tural, Ece, & Tural, 2018). Similar results have been reported for adsorption on CV dyestuff using acid-activated clay (Collins & Elijah, 2019) and potato skin (Lairini et al., 2017).

### Effect of Adsorbent Amount

CV dye solutions were prepared at pH: 6 and 10 $mgL^{-1}$ concentration. At 313 K, the quantity of adsorbent was studied with 0.05 g, 0.1 g, 0.2 g, 0.4 g, 0.6 g, 0.8 g, 1 g BK adsorbent. The results are shown in Figure 5.

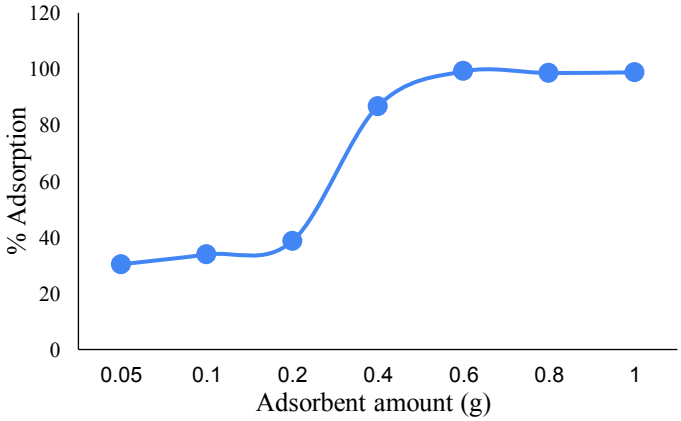


Figure 5. The effect of the amount of adsorbent on the BK adsorbent of the CV dyestuff (pH:6.0, concentration = 10 $mgL^{-1}$, temperature = 313 K)

The adsorption capacity is affected by the amount of adsorbent, which is an important parameter in determining the amount of adsorption (Kumari, Krishnamoorthy, Arumugam, Radhakrishnan, & Vasudevan, 2017). As the amount of adsorbent increases, so does the surface area of the adsorbent for CV dye molecules and the active sites for adsorption increase. This is because the CV dyestuff does not

saturate the active sites on the adsorbent surface. As the amount of adsorbent increases, so does the adsorption percentage. As a result, specific surface areas are associated with adsorption. It is understood that a specific amount of BK adsorbent can only adsorb a specific amount of dyestuff (Tural et al., 2018). Thus, the adsorption capacity of the dye rose as the amount of adsorbent increased and reached an equilibrium value of 0.6 g of adsorbent (Figure 5) (Bayramoglu, Altintas, & Arica, 2009; Kumari et al., 2017). The adsorbent comes into contact with less CV dyestuff after equilibrium, which means that the active sites in the adsorbent remain unsaturated. Therefore, the adsorption value decreased (Tural et al., 2018). The remaining studies were carried out 0.6 g of adsorbent.

### Effect of Interaction Time

The batch system was used to investigate the interaction time of CV dyestuff solution (70 $mgL^{-1}$, pH: 6.0) at 313 K temperature with BK adsorbent (0.6 g). The effect of interaction time (0, 1, 3, 5, 7, 10, 15, 25, 35, 45, 60, 75, 90 min) on BK adsorption on CV dyestuff is shown in Figure 6.

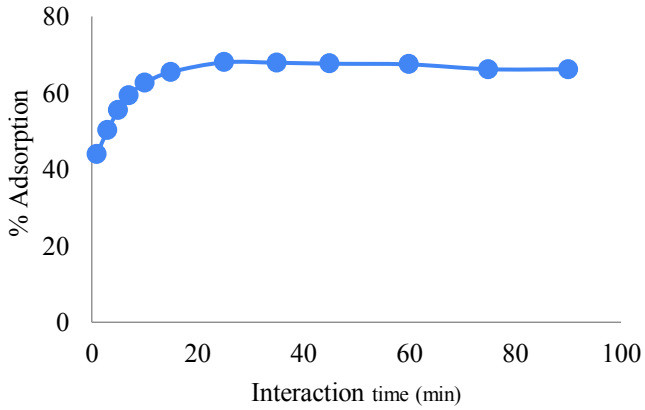


Figure 6. The effect of interaction time on the amount of adsorption (pH:6.0, adsorbent amount = 0.6 g, concentration = 70 $mgL^{-1}$, temperature = 313 K

As shown in Figure 6, the CV dye adsorption ability of BK adsorbent is rapid at the beginning, then slow adsorption occurs, and the adsorption reaches equilibrium at 25 min. The fact that the equilibrium is reached quickly shows that the BK adsorbent is very efficient for CV dye adsorption. The adsorption tendency of the CV dyestuff as a function of the interaction time revealed that the adsorption occured in two different stages. The first fast phase occurs in a very short amount of time, followed by the slow adsorption phase which takes a relatively longer amount of time. Slow adsorption is observed after reaching equilibrium, due to the reduction of vacancies when the binding sites reach saturation. As a result, the adsorption capacity did not change significantly. Furthermore, slow adsorption may be due to the intraparticle diffusion process dominating adsorption (Manzoor, Nadeem, Iqbal, Saeed, & Ansari, 2013; Shoukat, Bhatti, Iqbal, & Noreen, 2017; Ullah, Nadeem, Iqbal, & Manzoor, 2013). These results are in line with previous studies for different adsorbents as a function of contact time (El Haddad, Slimani, Mamouni, ElAntri, & Lazar, 2013; Mahamad, Zaini, & Zakaria, 2015; Ogata, Imai, & Kawasaki, 2015; Shoukat et al., 2017).

### Effect of Concentration

With a 25 minute interaction time, the adsorption of CV dyestuff on the BK adsorbent (0.6 g) at 313 K was investigated with solutions of various concentrations (10, 30, 50, 70 and 100 $mgL^{-1}$) (Figure 7).

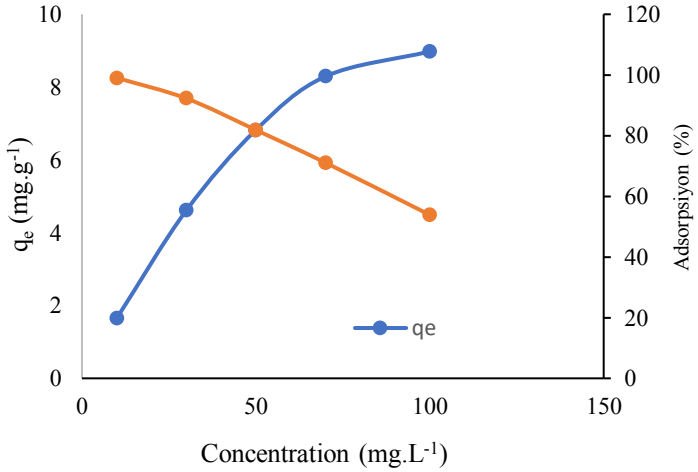


Figure 7. Effect of dye concentration on adsorption capacity (pH = 6.0, temperature = 313 K, amount of adsorbent = 0.6 g, interaction time: 25 min)

The adsorption capacity increases as the CV dyestuff concentration increases, whereas the adsorption percentage decreases. Adsorption of CV dyes is highly concentration-dependent. The amount of dye surrounding the active sites on the adsorbent surface is mostly responsible for the increase in $q_e$ values as CV

concentrations increase.. As the concentrations of CV dyestuff increases, more dyestuff surrounds the adsorption areas on the adsorbent surface, and therefore the CV is adsorbed more by the adsorbent. The percentage of adsorption decreases as the concentration increases, while the amount of adsorbent remains constant (Tural et al., 2018). The adsorbed percentage of dyestuff fell from 99 percent to 54 percent while the adsorption capacity of BK adsorbent increased from 1.65 $mgg^{-1}$ to 8.98 $mgg^{-1}$, (although the concentration increased from 10 $mgL^{-1}$ to 100 $mgL^{-1}$). Similar results have been previously reported by other researchers for different adsorbents before (R. Ahmad, 2009; Aksakal & Ucun, 2010; Idrissi, Miyah, Benjelloun, & Chaouch, 2016; Lairini et al., 2017; Shoukat et al., 2017). The maximum amount of CV dye adsorbed by the BK adsorbent is 8.98 mg.g-1 (Figure 7).

### Adsorption Isotherms

The balance between the CV dyestuff concentration remaining in the aqueous solution and the adsorbed CV dyestuff concentration was determined using Langmuir, Freundlich and Dubinin Radushkevich (D R) isotherm models.

### Langmuir Isotherm

It is calculated using the linear equation of the Langmuir isotherm in Equation 3.

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad (3)$$

$C_e$ ($mgL^{-1}$) is the equilibrium concentration, $q_e$ ($mgg^{-1}$) is the adsorption capacity at equilibrium, $q_m$ ($mgg^{-1}$) is the maximum adsorption capacity, $K_L$ ($Lmg^{-1}$) is the Langmuir adsorption constant. According to Langmuir isotherm theory, the adsorbate material assumes a single layer on a homogeneous adsorbent surface (Kireç, Alacabey, Erol, & Alkan, 2021). Correlation coefficients ($R^2$) of 0.9935, 0.9968 and 0.9972, respectively, were calculated for the CV dyestuff (Table 2).

In equation 4, starting concentration is $C_0$ ($mgL^{-1}$). In addition, the separation factor ($R_L$) is a unitless constant that is used to predict whether or not an adsorption process is favourable . RL values indicate that adsorption is unfavorable when ($R_L$>1), linear when ($R_L$=1), when favorable (0<$R_L$<1), and irreversible when ($R_L$=0) (Caliskan et al., 2011).

$$R_L = \frac{1}{1 + K_L C_O} \quad (4)$$

### Freundlich Isotherm

The linear equation of the Freundlich isotherm is given in equation 5 (Erol, 2016).

$$lnq_e = lnK_F + \frac{1}{n} lnC_e \quad (5)$$

The adsorption capacity is denoted by $K_F$ (Freundlich constant)), and n is the adsorption intensity. According to Freundlich, the adsorption sites on the surface of an adsorbent are heterogeneous, which means that they consist of different types of adsorption sites (Kireç et al., 2021).

### Dubinin-Radushkevich (D-R) Isotherm

The Dubinin-Radushkevich (D-R) isotherm was calculated using equations 6, 7, 8.

$$\ln q_e = \ln q_m - k\varepsilon^2 \quad (6)$$

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \quad (7)$$

$$E = (2k)^{-1/2} \quad (8)$$

$q_e$ is the quantity of CV dye adsorbed per unit BK adsorbent ($mgg^{-1}$), k is the the adsorption energy ($mol^2/kJ^2$) constant, $q_m$ is the DR adsorption capacity ($mgg^{-1}$), ε is the Polanyi potential ($kJmol^{-1}$), T is the temperature (K), and R is the gas constant (kJ/Kmol).

Equation 8's adsorption energy E ($kJmol^{-1}$) gives information about the adsorption mechanism. The adsoprtion process is classified as chemical, ion exchange or physical adsorption based on the determined E value. In the case of E < 8 $kJmol^{-1}$, the adsorption mechanism is realized by physical interactions. Regarding 8 < E < 16 $kJmol^{-1}$, the adsorption process is proceeded by ion exchange. E > 16 $kJmol^{-1}$ values indicate that chemical interactions are used to realize the adsorption mechanism (İhsan Alacabey, 2014).

Table 2. Langmuir, Freundlich and D–R isotherm constants

| | Langmuir | | | Freundlich | | | | D-R | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T(K) | $K_L$ ($Lmg^{-1}$) | $q_m$ ($mgg^{-1}$) | $R^2$ | n | 1/n | $K_F$ ($mgg^{-1}$) | $R^2$ | $q_m$ ($molg^{-1}$) | E ($kjmol^{-1}$) | $R^2$ |
| 298 | 0.2957 | 8.8797 | 0.9935 | 3.1870 | 0.3138 | 2.7117 | 0.9853 | 6.5180 | 2.6024 | 0.8523 |
| 313 | 0.4969 | 9.2842 | 0.9968 | 3.5165 | 0.2844 | 3.3974 | 0.9856 | 7.0329 | 3.7111 | 0.8750 |
| 323 | 0.6084 | 10.2475 | 0.9972 | 3.5731 | 0.2829 | 3.9085 | 0.9765 | 7.5570 | 4.3640 | 0.8609 |

Table 3. $R_L$ values for adsorption of CV onto BK adsorbent

| $C_0$ | 298 K | 313 K | 323 K |
|---|---|---|---|
| 10 | 0.2527 | 0.1675 | 0.1412 |
| 30 | 0.1013 | 0.0629 | 0.0519 |
| 50 | 0.0634 | 0.0387 | 0.0318 |
| 70 | 0.0461 | 0.0279 | 0.0229 |
| 100 | 0.0327 | 0.0197 | 0.0162 |

The adsorption was found to be suitable for the Langmuir model, as shown in Table 2. In other words, it demonstrates that the adsorption is monolayer and homogeneous (İhsan Alacabey et al., 2020; Erol, Tatar, Veyisoğlu, & Tokatlı, 2021). It was determined that the RL separation factors of $0.0162 \leq R_L \leq 0.2527$ for the CV dyestuff on the BK adsorbent were between 0 and 1, which indicated favourable adsorption (Table 3).

The Freundlich constant ($K_F$) varies between 2.7117 mg/g and 3.9085 mg/g, depending on the adsorption capacityand the temperature range examined.. Since the $K_F$ value increases with increasing temperature, the adsorption interaction is endothermic. The constant n determines the type of process: if n=1, the adsorption is linear; If n<1, adsorption is a chemical process; If n > 1, the adsorption takes place in a physical process (İhsan Alacabey, 2022). Thus, from Table 2, the n values for all the temperatures examined in this study was determined as 3.1870, 3.5165, and 3.5731. The most common condition is n>1 Any factor that causes dispersion of surface areas or a decrease in adsorbent-adsorbate interaction due to increased surface density can cause it. Values in the range of 1-10 show that CV dyestuff on BK adsorbent is adequate for adsorption and represents physical adsorption on the adsorbent (Shin & Kim, 2016).

As seen in Table 2, since the E value is less than < 8 kJmol$^{-1}$, the adsorption process is physical.

## Adsorption Thermodynamics

Gibbs free enthalpy ($\Delta G^o$), adsorption entropy ($\Delta S^o$) and adsorption enthalpy ($\Delta H^o$) all play an important role in determining the adsorption type in thermodynamics. It is calculated by using equations 9, 10, 11, 12.

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (9)$$

$\Delta G°$ is free energy change (kJmol$^{-1}$), $\Delta S^o$ is Entropy change (kJmol$^{-1}$K$^{-1}$), $\Delta H°$ is enthalpy change (kJmol$^{-1}$T$^{-1}$), T is the temperature (K).

$K_c$ is calculated using equation 10 in the calculation of Gibbs free energy in the adsorption process.

$$K_c = C_a/C_e \quad (10)$$

$C_a$ is the concentration of the substance adsorbed by the BK adsorbent (mgL$^{-1}$), Ce is the concentration of the substance remaining in the solution (mgL$^{-1}$), and $K_c$ is the equilibrium constant,

The cut-off point of the graph of $K_c$ drawn against the initial CV dyestuff concentrations ($C_0$) yields the $K_c$ value. Afterwards, if the $K_c^o$ value is written in equation 11, the Gibbs free energy of adsorption is found. The Gibbs free energy determines the degree of the adsorption process' spontaneity.

$$\Delta G^0 = -RT\ln Kc^0 \quad (11)$$

$$\ln K_c{}^o = \frac{\Delta S^o - \Delta H^o}{R} \times \frac{1}{T} \quad (12)$$

R is the gas constant (8.314 J mol$^{-1}$ K$^{-1}$).

Using equation 12, $\Delta H^o$ and $\Delta S^o$ are calculated from respectively the slope and the cut-off point of the graph of lnKc0 drawn against the 1/T value.

Table 4. Thermodynamic parameter values for adsorption of CV dyestuff on BK adsorbent

| $C_0$ | ΔH°, kj/mol | ΔS°, j/mol | ΔG°, kj/mol | | |
|---|---|---|---|---|---|
| | | | 298 K | 313 K | 323 K |
| **10** | 39.68 | 165.16 | -9.52 | -12.07 | -13.63 |
| **30** | 33.87 | 127.98 | -4.17 | -6.45 | -7.30 |
| **50** | 27.24 | 101.23 | -3.11 | -3.92 | -5.78 |
| **70** | 16.19 | 59.56 | -1.60 | -2.34 | -3.12 |
| **100** | 11.60 | 38.85 | -0.04 | -0.40 | -1.05 |

$\Delta H^o$, $\Delta G^o$ and $\Delta S^o$ values are given in table 4. $\Delta H^o$ values were determined to be in the range of 39.68 to 11.60 kJ mol$^{-1}$. Positive $\Delta H^o$ values indicate that the adsorption is endothermic. Also, the adsorption enthalpy is less than 40 kJ mol$^{-1}$, which indicates that the physical mechanism is controling the adsorption processes (Caliskan et al., 2011; Sogut & Caliskan, 2017). In addition, ΔSo values were found to range from 165.16 to 38.8 JK$^{-1}$mol$^{-1}$. Positive values of $\Delta S^o$ indicate an increase in randomness at the solid-liquid interface during CV adsorption onto the adsorbent (İ Alacabey, 2006). Negative $\Delta G^o$ values show that adsorption occurs spontaneously (Alkan, Kul, Alacabey, & Erol, 2014). These values decrease as the tempereature increases temperature. Also, adsorption is better obtained at higher temperatures (Caliskan et al., 2011).

## Conclusion

The removal of CV dyestuff in the aqueous solution on the BK adsorbent was examined using the batch adsorption method.

1. The Langmuir model provides the optimal adsorption, which is monolayer and homogeneous, based on the equilibrium adsorption data for CV aqueous solution.

2. The $K_F$ value increases with increasing temperature in the Freundlich model, which indicates that the adsorption interaction is endothermic. The n value in the range of 1-10 indicates that it is suitable for CV dyestuff adsorption on the BK adsorbent and represents the physical adsorption on the adsorbent.

3. The fact that the E value is less than 8 kJmol$^{-1}$ in the Dubinin-Radushkevich (D-R) model indicates that the adsorption process is physical.

4. The adsorption enthalpy values are less than 40 kJ mol-1 in all cases studied, confirming that the adsorption process is regulated by a physical rather than a chemical mechanism (Sogut & Caliskan, 2017).

This study showed that the adsorption process for Crystal Violette removal with Bardakçı Clay, endothermic, both physically and spontaneously. As a

result, it shows that Bardakçı Clay can be used as a low-cost, effective adsorbent for CV removal.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

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Ethics committee approval is not required.

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# Leaf and root-growth characteristics contributing to salt tolerance of backcrossed pepper (*Capsicum annuum* L.) progenies under hydroponic conditions

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## Abstract
The aim of this study was to determine the genotypic differences in salt tolerance of third back-crossed peeper progenies and their respective parents through examining the changes in the shoot growth at agronomical, root growth at morphological and leaf development at physiological levels under salt stress. A hydroponic experiment was conducted by using an aerated Deep-Water Culture (DWC) technique in a controlled growth chamber of Erciyes University, Agricultural Faculty in Kayseri, Turkey. Five pepper plants (BC3-1, BC3-2, BC3-3, BC3-4, BC3-5) were selected from the third backcrossed (BC3) progenies of Sena and Kopan. Plants were grown in 8 L pots filled continuously aerated nutrient solution under at two electrical conductivity (EC) levels (control at 1.0 dS $m^{-1}$ and salt at 8.0 dS $m^{-1}$) in RBD design with four replications for six weeks. Significant reductions in leaf, shoot and root fresh and dry biomass productions, total leaf area, total root length, and total root volume of pepper plants were recorded under hydroponic salt stress. On the other hand, significant differences in salt tolerance among backcrossed peeper progenies and their respective parents existed. Particularly the progeny of BC3-3 was more tolerant characterized to salinity than the other progenies of third backcrossed and their respective parents. This was highly associated with vigorous root growth (root fresh and dry weight, total root length and volume) and photosynthetically active leaves (total leaf area, leaf chlorophyll index, chloride exclusion) under hydroponic salt stress. These traits could be useful characters to select and breed salt-tolerant pepper varieties for sustainable agriculture in the future.

## Keywords
Back crossing, *Chile penguin*, Hydroponic system, Salinity, SPAD, Total root length

## Introduction
Salinity is the one of the main abiotic stress factors that adversely affects crop growth and productivity by decreasing yield and quality, particularly in arid and semi-arid regions (Huez-López et al., 2011). Although, some advanced management practices were developed in recent decades, currently, more than one billion hectares of land, accounting for approximately 25% of the global land area is being affected by salinity. Due to natural salinization or unsuitable irrigation practices, this area is increasing by up to 10 million hectares of land every year. In Turkey, 4 million hectares of land was affected by salinization (Abidalrazzaq Musluh Al Rubaye et al., 2021). Salinity stress is rising in agricultural areas owing to numerous factors including rising in sea levels, low-level rainfall, high-level evaporation, climate change, excessive irrigation without proper drainage in lands, and underlying rocks rich in harmful salts etc. (Kumar et al., 2013). In addition, if the current scenario of salinity stress would continue, 50% of the land currently cultivated for agriculture may be lost by 2050 [Wang et al 2003]. Most of the crop plants are sensitive to salinity and thus under excessively saline conditions the crops usually exhibit shorter life cycles or limited plant growth and diminished biomass production (Abidalrazzaq Musluh Al Rubaye et al., 2021). It has been demonstrated that salinity stress causes various major problems in crop plants such as intercellular damage and metabolic disturbance (Chartzoulakis and Klapaki, 2000),

reduction in stomatal conductance, transpiration rate and net photosynthesis (Lycoskoufis et al., 2005), water stress in source leaves and carbohydrate shortage in sink leaves (Kurtar et al., 2016), ion toxicity by excessive $Na^+$ and $Cl^-$ accumulation and reduction of $K^+$ uptake (Wu et al., 2013), and inhibition in root growth, nutrient uptake (Colla et al., 2013).

Pepper (*Capsicum annuum* L.) is an important horticultural crop in the world due to its economic significance and the nutritional value of fruits. Global pepper production has increased over the last 20 years from 17 to 36 million tons (Mt), and the cultivated area has expanded by about 35% particularly in arid and semiarid regions (Tripodi and Kumar, 2019). Pepper is a warm-season crop possessing vulnerability features to abiotic and biotic factors. Pepper plants are considered moderately sensitive, sensitive, or highly susceptible to salinity stress (Penella et al., 2017). Dry pepper cultivation in Turkey is generally carried out in arid and semi-arid regions in the southeastern part of the country. As mentioned above, one of the important problems in arid and semi-arid regions is continuing salinization in the soil. For this reason, the breeding and releasing of salt stress-tolerant varieties are important for the sustainability of dry pepper production in these areas. To avoid or reduce salt stress impacts and hinder yield losses for sustainable pepper production, integrated salt management strategies that take into consideration improved soil and crop management practices are necessary. Moreover, another way is to improve the salinity tolerance of high-yielding salt susceptible pepper cultivars would be applying an interspecific hybridization breeding technique.

In the past decades to enhance salinity tolerance in crops, several strategies such as conventional selection and breeding techniques have been recommended and applied (Shannon, 1998). Interspecific hybridization has been used to introgress useful traits from wild and related species into cultivated varieties in many Solanaceous crops, particularly in terms of pest and disease resistance. The Capsicum genus involve 27 species, 5 domesticated and 22 wild-type species. As genetic resources for breeding enhanced *Capsicum annuum*, wild and related Capsicum species are beneficial not only breeding disease resistance, although additionally for improving the yield, nutritional quality, and adaptation to abiotic stresses (Yoon et al., 2006). However, no comprehensive hydroponic studies were found in the literature that aimed to search the answer to the most critical questions of which agronomical, physiological and root morphological characteristics are contributing to salt tolerance, and which are the consistent traits for selecting salt-tolerant pepper genotypes. Therefore, the aim of this study was to determine the genotypic differences in salt tolerance of third backcrossed (BC3) peeper progenies and their respective parents [Sena (*Capsicum annuum* L.) and Kopan (*Chile penguin*)] through examining the changes in the shoot growth at agronomical, root growth at morphological and leaf development at physiological levels under hydroponic salt stress. This approach will help to identify plant traits that are suitable for the selection and breeding of salt-tolerant pepper varieties for sustainable agriculture in the future.

## Materials and Methods

### Plant material, treatments, and experimental design

This study was carried out in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. A hydroponic experiment was conducted by using an aerated deep-water culture (DWC) technique in a fully automated climate chamber. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65–70% and about 390 µmol $m^{-2}$ $S^{-1}$ photon flux was sup-plied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. The plant materials used were backcross lines obtained through consecutive backcrossing for three generations of Sena (*Capsicum annuum* L.) and Kopan (*Chile penguin*). Five selected BC3 plants (BC3-1, BC3-2, BC3-3, BC3-4, BC3-5) and their parents screened for salinity tolerance at 8 $dS \cdot m^{-1}$. The seeds used in the experiment were produced by selfing from the parents and the plants selected from the backcross population. Kopan has small, hot pepper fruits that had lower strength for separation from fruit pedicel. Sena has fruits that are approximately 10 cm in length and 2.5 cm in diameter and appropriate for pepper powder and dry chili pepper production. The seeds were sown in 72-cell polystyrene trays (W 280 × L 540 × H 45 mm, IBK Iklim Bahce Co., Ltd., Turkey) filled with a mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). To promote germination, the plug trays were wrapped with vinyl chloride resin film and then placed in a germination chamber at 28°C. After four days, the germinated seedlings were moved to a greenhouse. Seedlings were watered daily. When the seedlings developed three or four true leaves, they were transplanted to plastic pots after the root system of the plants was carefully washed in distilled water to clean the substrate.

For salinity treatments two electrical conductivity (EC) levels (control at 1.0 dS $m^{-1}$ and salt at 8.0 dS $m^{-1}$) were applied two weeks after transplantation. Each pot was filled with 8 L cultivation solution that was aerated by an air pump. The nutrient solution contained 1.5 mM calcium nitrate ($Ca(NO_3)_2$,) 250 µM monopotassium phosphate ($KH_2PO_4$), 500 µM potassium sulfate ($K_2SO_4$), 325 µM magnesium sulfate ($MgSO_4.7H_2O$), 50 µM sodium chloride (NaCl). Micronutrients were 80 µM iron (Fe) (III)- ethylenediaminetetraacetic acid (EDTA)- sodium (Na), 0.4 µM manganese sulfate ($MnSO_4$), 0.4 µM zinc sulfate ($ZnSO_4$), 0.4 µM copper sulfate ($CuSO_4$), 8 µM boric acid ($H_3BO_3$), 0.4 µM sodium molybdate ($Na_2MoO_4$). Solutions were replaced completely every week in the first two weeks. In hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to final harvest was almost six weeks. The experiment was in a completely randomized block design with three replications and four plants in each replication.

### Biomass Determination

At the end of the experiment, the biomass of the aerial part of the plants was deter-mined. Pepper plants were harvested by separating them into stems, leaves, and roots and weighed for fresh biomasses determination. Immediately afterward, their tissues were dried in a forced-air oven at 70 ◦C for 72 h for dry

biomass determination until the stable weight was reached. And then they were weighed on an electronic digital scale. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems) and was expressed in g plant$^{-1}$. Root: shoot ratio was calculated by dividing the root dry weight by the shoot dry weight.

### Root Morphological Measurements

The plant root morphological parameters such as total root length (m plant$^{-1}$), total root volume (cm$^3$ plant$^{-1}$) and average root diameter (mm) were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc., Québec, QC G1V 1V4, Canada) in combination with a recording device of Epson Expression 11000XL scanner (Long Beach, CA, USA). It was recorded as cm plant-1, and then converted to m plant$^{-1}$.

### Leaf Physiological Measurements

In the hydroponic experiment, the total leaf area of the plants was measured destructively during the harvesting process by using leaf-area meter (LI-COR Model 3100, LI-COR. Inc., Lincoln, NE, USA). Total leaf area was recorded in centimeter square (cm$^2$). Prior to harvest, non-destructive measurement of the leaf chlorophyll content (SPAD) was done in a controlled growth chamber by using the Minolta SPAD-502 chlorophyll meter. During the growth period, the leaf chlorophyll content measurement was performed on the youngest fully expanded leaves (3rd–4$^{th}$ leaf from the apex) of whole plants, using four replicate leaves per treatment in the third and fifth week of the vegetation period. The measurements were carried out at the temperature of 25/22°C (average day/night temperatures), the relative humidity of 65–70%. The supplied photon flux in the growth chamber was almost 390 µmol m$^{-2}$ S$^{-1}$ with an intensity of 16/8h (light/dark) photoperiod. All SPAD readings were carried out between 09:00 and 12:00 HR.

### Leaf Ion Determination

Dried plant tissues (leaf) were ground separately in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 20-mesh screen; then 0.5 g of the dried plant tissues were analyzed for chloride concentration ($Cl^-$). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

### Statistical Analysis

All measured physiological and morphological parameters were analyzed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of genotypes and salt and their interactions on the variables analyzed. Levels of significance are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns means not significant. Differences between the treatments were compared using Duncan's Multiple Test ($p < 0.05$).

## Results and Discussion

### Biomass Production and Partitioning

To evaluate whether plants provided a similar growth rate after NaCl treatment, fresh and dry biomass were measured in both roots and aerial organs at final harvest. According to the results shown in Table 1 generally, the growth and development of the pepper plants were negatively affected by increasing the salt level of the nutrient solutions. Plant growth significantly decreased as the NaCl concentration in the nutrient solution increased. The growth performance of the pepper plants was dependent on genotype and salinity. Fresh matter of leaf, shoot and root were significantly ($p$ <0.001) affected by genotype, different salt levels and genotype × salt interaction (Table 1). Fresh matter of leaf, shoot and root decreased by different rates in each genotype with the salt application. Leaf fresh matter ranged between 9.10-16.27 g plant$^{-1}$ under control conditions (1 dS m$^{-1}$) and between 4.22-10.92 g plant$^{-1}$ under saline conditions. Compared to the control plants, 8 dS m$^{-1}$ NaCl decreased the leaf fresh matter of BC3-3 and BC3-5 plants by 21.5 and 71.9%, respectively.

Regarding shoot fresh matter, it ranged between 10.60-20.00 g plant$^{-1}$ under control conditions (1 dS m$^{-1}$) and between 4.99-13.62 g plant$^{-1}$ under saline conditions. Under non-saline conditions, BC3-2 has a significantly higher leaf and shoot fresh matter, whereas Sena has significantly lower leaf and shoot fresh matter than other backcross plants. Salt stress decreased the shoot fresh matter of BC3-3 and BC3-5 plants by 26.3 and 74.5%, respectively as compared to unstressed plants. Regarding root fresh matter, BC3-1 plants developed a higher root system than respective parents and their progenies of third backcrosses under non-saline conditions. Root fresh matter ranged between 5.41-13.72 g plant$^{-1}$ under control conditions (1 dS m$^{-1}$) and between 2.90-9.30 g plant$^{-1}$ under saline conditions. Salt-stress caused a decrease in leaf, shoot and root fresh matter in all treated respective parents and their progenies of third backcrosses studied. 8 dS m$^{-1}$ NaCl treatment decreased the root fresh matter of BC3-3 and BC3-4 plants by 19.1 and 56.0%, respectively compared to control plants. BC3-3 had a significantly higher leaf and root fresh matter, whereas BC3-5 has significantly lower leaf and shoot fresh matter than other respective parents and their progenies of third backcrosses under saline conditions. Sena had a significantly lower root fresh matter under saline conditions.

Results of the leaf, shoot and root dry matter of the pepper plants grown in different salt levels (1 dS m$^{-1}$ and 8 dS m$^{-1}$) at the end of the growing cycle were shown in Table 2. Dry matter of leaf, shoot and root were significantly ($p$ <0.001) affected by genotype, different salt levels and genotype × salt interaction. Salt stress applied through the nutrient solution significantly inhibited plant growth, reduce plant mass (Table 2), total leaf area (Fig. 1A) and leaf chlorophyll content (Fig. 1B). The exposure of pepper plants to NaCl salinity (8 dS m$^{-1}$) resulted in a marked suppression of the leaf, shoot and root dry matter. Salt stress adversely affects the growth and development of pepper plants, and the results of our study confirm that all growth variables of pepper genotypes drastically decreased with NaCl treatment. Under non-saline conditions, leaf, shoot and root dry matter in BC3-1 were found to be significantly higher than respective parents and their progenies of third backcrosses. On the other hand, under saline conditions, BC3-1, BC3-2, BC3-3, and BC3-4 had significantly higher leaf dry matter; though, BC3-5, Kopan and Sena had significantly lower leaf dry matter. Compared to the unstressed plants, salt stress decreased the leaf dry matter of BC3-4 and BC3-5 plants by 24.5

and 68.3%, respectively compared to control plants. There was a significant difference between pepper cultivars for shoot dry matter. The maximum accumulation of dry matter of the shoot was in BC3-1 under saline conditions, although the lowest accumulation of dry matter of the shoot was in Kopan. Salt stress decreased shoot dry matter from 26.5% in BC3-3 to 64.9% in BC3-5 as comparing with unstressed plants.

The root dry matter varied from 0.86 in BC3-3 to 0.25 in Sena of 8 dS $m^{-1}$. 8 dS $m^{-1}$ NaCl decreased the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively than control plants.

Table 1. The effects of different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) on fresh matter of leaf, shoot and root of pepper plants

| | Leaf fresh matter (g $plant^{-1}$) | | | Shoot fresh matter (g $plant^{-1}$) | | | Root fresh matter (g $plant^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Control | Salt | % R | Control | Salt | % R | Control | Salt | % R |
| BC3-1 | 15.88 b | 10.60 B | 33.3 | 19.61 b | 13.62 A | 30.5 | 13.72 a | 7.93 C | 42.2 |
| BC3-2 | 16.27 a | 10.38 C | 36.2 | 20.00 a | 12.69 C | 36.6 | 13.09 b | 8.95 B | 31.7 |
| BC3-3 | 13.90 d | 10.92 A | 21.5 | 17.52 d | 12.92 B | 26.3 | 11.49 c | 9.30 A | 19.1 |
| BC3-4 | 11.90 e | 7.81 D | 34.3 | 17.72 c | 10.22 D | 42.3 | 10.00 d | 4.40 D | 56.0 |
| BC3-5 | 15.02 c | 4.22 G | 71.9 | 19.60 b | 4.99 F | 74.5 | 8.98 e | 3.99 E | 55.6 |
| Kopan | 10.72 f | 4.31 F | 59.8 | 11.79 e | 5.03 F | 57.3 | 6.60 f | 3.60 F | 45.4 |
| Sena | 9.10 g | 4.41 E | 51.5 | 10.60 f | 5.63 E | 46.9 | 5.41 g | 2.90 G | 46.3 |
| F-Test | | | | | | | | | |
| Genotype | | *** | | | *** | | | *** | |
| Salt | | *** | | | *** | | | *** | |
| Genotype × Salt | | *** | | | *** | | | *** | |

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 2. The effects of different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) on dry matter of leaf, shoot and root of pepper plants

| | Leaf dry matter (g $plant^{-1}$) | | | Shoot dry matter (g $plant^{-1}$) | | | Root dry matter (g $plant^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Control | Salt | % R | Control | Salt | % R | Control | Salt | % R |
| BC3-1 | 1.74 a | 1.12 A | 35.3 | 2.16 a | 1.45 A | 32.8 | 1.26 a | 0.74 C | 41.6 |
| BC3-2 | 1.61 b | 1.09 A | 32.3 | 1.95 b | 1.33 B | 31.9 | 1.21 b | 0.83 B | 31.3 |
| BC3-3 | 1.46 d | 1.09 A | 25.6 | 1.74 c | 1.28 B | 26.5 | 1.04 c | 0.86 A | 17.3 |
| BC3-4 | 1.43 d | 1.08 A | 24.5 | 1.89 b | 1.36 AB | 28.0 | 0.92 d | 0.41 D | 55.3 |
| BC3-5 | 1.52 c | 0.48 B | 68.3 | 1.94 b | 0.68 C | 64.9 | 0.82 e | 0.36 E | 56.2 |
| Kopan | 1.18 e | 0.46 B | 61.4 | 1.28 d | 0.53 D | 58.3 | 0.61 f | 0.33 F | 46.4 |
| Sena | 0.97 f | 0.52 B | 46.6 | 1.14 e | 0.60 CD | 47.2 | 0.50 g | 0.25 G | 49.6 |
| F-Test | | | | | | | | | |
| Genotype | | *** | | | *** | | | *** | |
| Salt | | *** | | | *** | | | *** | |
| Genotype × Salt | | *** | | | *** | | | *** | |

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

## Total Leaf Area and Leaf Chlorophyll Content

The results shown in Figure 1A and 1B showed that the negative salinity effect resulted in a clear decrease in total leaf area and leaf chlorophyll content (SPAD) by increasing salt concentrations. Total leaf area and SPAD were significantly ($p$ <0.001) affected by genotype, different salt levels and genotype × salt interaction. Total leaf area was significantly decreased by increasing salt level of the nutrient solution. Salt treatment with increasing concentrations of the nutrient solution negatively affected plant growth causing significant decreases in total leaf area and leaf chlorophyll content. Total leaf area ranged between 358-604 $cm^2$ $plant^{-1}$ in control conditions and between 159-386 $cm^2$ $plant^{-1}$ in the saline conditions. Significantly higher total leaf area formation was observed in BC3-2, whereas significantly lower total leaf area formation was observed in Sena. On the other hand, under salt stress conditions, BC3-3 had significantly higher total leaf area formation as compared to respective parents and their progenies of third back-crosses. Significantly lower total leaf area formation was observed in BC3-5 and Sena (Figure 1A). 8 dS $m^{-1}$ NaCl decreased the total leaf area of BC3-3 and BC3-5 plants by 28.2 and 72.9%, respectively than control plants.

Based on the SPAD, the supply of saline nutrient solution to pepper plants restricted markedly the rate of SPAD during the whole growing period, and the suppression was consistently more profound under salinity. Leaf chlorophyll content ranged between 33.2-51.9 in control conditions and 32.7-53.8 in saline conditions. At control conditions, BC3-4 had a higher SPAD value and was significantly different from respective parents and their progenies of third

backcrosses. Under saline conditions, the highest SPAD value was observed at BC3-2 and BC3-5 and significantly different from respective parents and their progenies of third backcrosses (Figure 1B). The lowest SPAD value was determined in the Kopan pepper genotype.

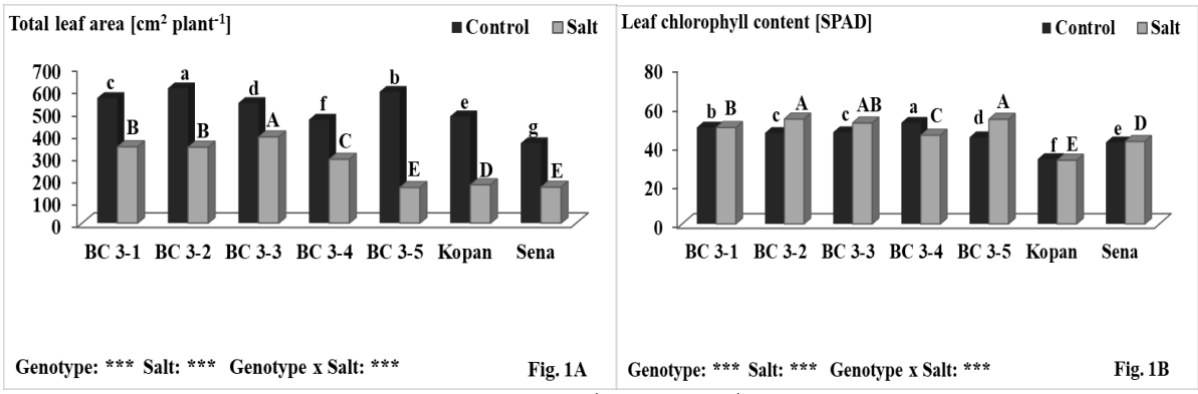


Figure 1. The effects of different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) on total leaf area (A), and leaf chlorophyll content (B) of pepper plants.

Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

## Root Morphological Development and Root Architecture

The results of the total root length, total root volume and average root diameter at the end of the growing cycle of pepper plants grown in different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) were shown in Table 3. Total root length, total root volume and average root diameter were significantly ($p$ <0.001) affected by genotype, different salt levels and genotype × salt interaction. The root growth of pepper plants was severely restricted at a salinity level of 8 dS $m^{-1}$. Pepper plants under 1 dS $m^{-1}$ salt concentrations produced higher total root length and total root volume than plants grown under 8 dS $m^{-1}$ salt concentrations. The effects of 8 dS $m^{-1}$ NaCl on total root length in all pepper plants, respectively, showed a similar trend as root fresh weight. Under control conditions, significantly greater total root length was produced at progenies of third backcrosses BC3-2; however significantly lower total root length was produced at Sena.

The total root length was ranked between 8649 m $plant^{-1}$ and 3538 m $plant^{-1}$ under control conditions. Compared to the unstressed plants, salt stress decreased the total root length of BC3-3 and BC3-4 plants by 7.8 and 61.3%, respectively compared to control plants. It was ranked from 6361 m $plant^{-1}$ and 2283 m $plant^{-1}$ under salinity. The progenies of third backcrosses of BC3-3 have significantly higher total root length, while BC3-4 produced significantly lower total root length (Table 3). Regarding total root volume, it was ranked between 4.33 $cm^3$ $plant^{-1}$ and 3.00 $cm^3$ $plant^{-1}$ under non-saline conditions. On the other hand, under saline conditions it was ranked from 4.14 $cm^3$ $plant^{-1}$ to 1.77 $cm^3$ $plant^{-1}$. 8 dS $m^{-1}$ NaCl decreased the total root volume of BC3-1 and BC3-4 plants by -30.2 and 49.9%, respectively than control plants.

The maximum root volume found were 4.33 $cm^2$ $plant^{-1}$ in Kopan grown under control conditions whereas B3-3 plant produced the highest root volume with 4.14 g under salt stress (Table 3). Concerning average root diameter, it was ranked among 0.336 mm and 0.223 mm under non-saline conditions; while, under saline conditions it was ranked among 0.353 mm and 0.288 mm.

Table 3. The effects of different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) on total root length, total root volume and average root diameter of pepper plants

| | Total root length (m $plant^{-1}$) | | | Total root volume ($cm^3$ $plant^{-1}$) | | | Av. root diameter (mm) | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Control | Salt | % R | Control | Salt | % R | Control | Salt | % R |
| BC3-1 | 7684 b | 5365 C | 30.2 | 3.00 d | 3.91 B | -30.2 | 0.223 g | 0.305 D | -36.6 |
| BC3-2 | 8649 a | 5369 B | 37.9 | 3.75 b | 3.66 C | 2.4 | 0.235 f | 0.295 E | -25.4 |
| BC3-3 | 6896 c | 6361 A | 7.8 | 3.46 c | 4.14 A | -19.7 | 0.253 e | 0.288 E | -13.9 |
| BC3-4 | 5896 d | 2283 G | 61.3 | 3.53 bc | 1.77 G | 49.9 | 0.276 d | 0.314 D | -13.8 |
| BC3-5 | 5856 e | 2461 F | 58.0 | 3.75 b | 2.08 F | 44.5 | 0.286 c | 0.328 C | -14.9 |
| Kopan | 5419 f | 2773 D | 48.8 | 4.33 a | 2.61 E | 39.8 | 0.319 b | 0.346 B | -8.5 |
| Sena | 3538 g | 2762 E | 22.0 | 3.14 d | 2.71 D | 13.7 | 0.336 a | 0.353 A | -5.2 |
| F-Test | | | | | | | | | |
| Genotype | | *** | | | *** | | | *** | |
| Salt | | *** | | | *** | | | *** | |
| Genotype × Salt | | *** | | | *** | | | *** | |

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Salinity significantly increased average root diameter in all tested respective parents and their progenies of third backcrosses; furthermore, a significantly higher average root diameter was produced at by recurrent parent Sena.

### Leaf Ion Determination

Results of leaf $Cl^-$ composition of pepper plants grown in different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) at the end of the growing cycle were presented in Figure 2. Leaf $Cl^-$ composition was significantly ($p$ <0.001) affected by genotype, different salt levels and genotype × salt interaction. Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Pasternak, 1987). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. The $Cl^-$ concentration in leaves increased with a higher NaCl concentration. Under saline conditions, Kopan accumulated the highest $Cl^-$ levels in leaves.

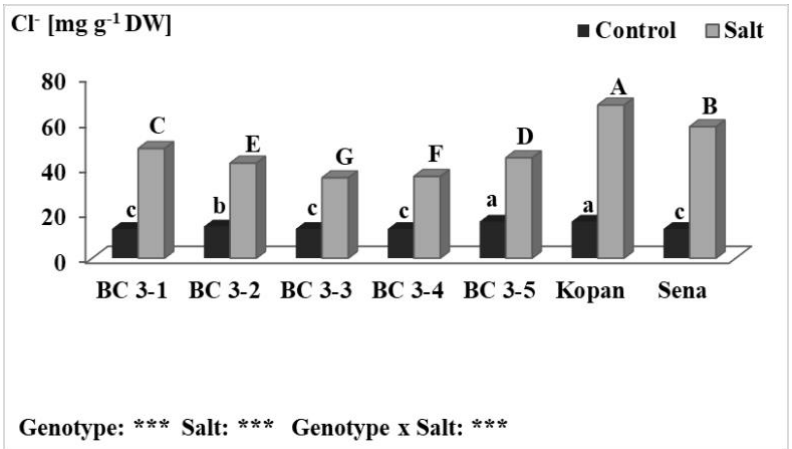


Figure 2. The effects of different salt levels (1 dS $m^{-1}$ and 8 dS $m^{-1}$) on leaf $Cl^-$ composition of pepper plants. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

## Discussion

To evaluate whether plants provided a similar growth rate after NaCl treatment, fresh and dry biomass were measured in both roots and aerial organs at final harvest. Our results showed that the growth, crop biomass and physiology of pepper plant decreased under salt stress. Compared to the control plants, 8 dS $m^{-1}$ NaCl decreased the leaf fresh matter of BC3-3 and BC3-5 plants by 21.5 and 71.9%, respectively. The decline in fresh matter of leaves in response to salt stress has been studied for other crops and different reductions rate have been reported depending on salt stress and genotype (Penella et al., 2017). It is known that pepper is a salt-sensitive plant species (Navarro et al., 2006). It is also well-known that salinity stress reduces plant growth and that there are differences among cultivars with peppers (Penella et al., 2017). The lower growth rate of the plant is associated with the end of new leaf expansion and limited leaf growth by slowing the cellular division. Bojórquez-Quintal et al (2014) studying the tolerance mechanisms to salt in chili habanero (*Capsicum chinense* Jacq.) plants in two varieties that exhibit different sensibilities to the salt stress, between them the 'Rex' variety, more tolerant than the 'Chichen-Itza' variety testify a concentration of 150 mM of NaCl through seven days for a culture in hydroponic conditions, and observed high impact on the growth of the two varieties with significant reduction of dry and fresh weight induced by NaCl in both the genotypes, in the fresh weight the reduction was greater in 'Chichen-Itza' (75%), than the reduction in the 'Rex' variety (50%).

Several studies have indicated that shoot and root growth in most horticultural crops, including cucumber, tomato, watermelon, pepper, and citrus, are inhibited by elevated NaCl concentrations in the soil or growth medium solution (Gong et al., 2013).

The reduction in plant growth under salt stress conditions could be attributed to the decline in osmotic potential because of excess concentration of $Na^+$ and $Cl^-$ ions in the root zone resulting in a nutritional imbalance (Pasternak, 1987). Also, inhibitory effects of salinity on vegetative growth may be because of the diversion of energy from growth to exclude $Na^+$ uptake and synthesis of compatible solutes to maintain cell turgor under hyperosmotic saline conditions (Munns and Tester, 2008).

Salt stress applied through the nutrient solution significantly inhibited plant growth, reduce plant mass (Table 2), total leaf area (Fig. 1A) and leaf chlorophyll content (Fig. 1B). The exposure of pepper plants to NaCl salinity (8 dS $m^{-1}$) resulted in a marked suppression of the leaf, shoot and root dry matter. Salt stress decreased shoot dry matter from 26.5% in BC3-3 to 64.9% in BC3-5 and the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively as comparing with unstressed plants. This can be associated with salt stress which can cause morphological, physiological and biochemical alteration in critical levels, interfering with the absorption and transportation of water and nutrients to the plant (Ulas et al., 2020). The decline in plant growth under salinity has also been demonstrated in other studies in pepper (Abidalrazzaq Musluh Al Rubaye et al., 2021), melon (Ulas et al., 2019; Ulas et al., 2020), cucumber (Colla et al., 2012), tomato (Gong et al., 2013), lettuce (Lucini et al., 2015) and pepino (Ulas, 2021) grown hydroponically under greenhouse conditions.

The negative salinity effect resulted in a clear decrease in total leaf area and leaf chlorophyll content

(SPAD) by increasing salt concentrations (Figure 1A and 1B). Total leaf area were significantly decreased by increasing salt level of the nutrient solution. Variability in SPAD chlorophyll content due to abiotic stresses such as water and salt stresses were reported in earlier work, and the use of suitable genotypes to provide suitable environmental conditions for the crop was suggested (Ahmed and Hassan, 2015). Salt treatment with increasing concentrations of the nutrient solution negatively affected plant growth causing significant decreases in total leaf area and leaf chlorophyll content. Our results are inconsistent with Sagi et al., (1997) who observed the adverse effects of salinity stress on leaf area. It has been reported that, the decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of sweet pepper, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis and inhibition of PSII activity. Reduction in leaf area is caused by ion accumulation in the leaves, particularly the old ones. Leaf chlorophyll content is often well correlated in salt-stressed plants (Gong et al., 2013). Changes in chlorophyll content may be associated with genotypes, stress period and stress intensity. Özdemir et al., (2016) stated that an increase in chlorophyll degradation or the decrease in the synthesis of chlorophyll may affect a decline in chlorophyll content.

The root growth of pepper plants was severely restricted at a salinity level of 8 dS $m^{-1}$ (Table 3). Pepper plants under 1 dS m-1 salt concentrations produced higher total root length and total root volume than plants grown under 8 dS m-1 salt concentrations. The effects of 8 dS $m^{-1}$ NaCl on total root length in all pepper plants, respectively, showed a similar trend as root fresh weight. Salinity significantly increased average root diameter in all tested respective parents and their progenies of third backcrosses. Based on the study by Dölarslan and Gül (2012) transpiration and respiration as well as water uptake and root development decreased under saline conditions. Likewise in our study, Abidalrazzaq Musluh Al Rubaye et al., (2021) stated that salt stress caused a decline in total root length depending on genotypes as compared to control plants in pepper plants.

Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Pasternak, 1987). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. The $Cl^-$ concentration in leaves increased with a higher NaCl concentration (Figure 2). These results are in agreement with other studies explaining that plants suffer from ionic imbalance and nutrient deficiency under salt stress (Abidalrazzaq Musluh Al Rubaye et al., 2021). It is important to sustain ion homeostasis to prevent toxic accumulation, as plants come into contact with salt. Plants survive with this situation by various mechanisms that can contribute to salt tolerance, some of which are very well documented in the bibliography (Isayenkov and Maathuis, 2019). It is well documented that $Cl^-$ may decline the leaf chlorophyll concentration and the activity of RuBP carboxylase without a corresponding decline in the concentration of this enzyme, which is accompanied by suppression of leaf photosynthesis. Efficient intracellular compartmentation of $Cl^-$ may arise in salt tolerant plant species, thus defending chloroplasts from undesirably high $Cl^-$ concentrations. Though, salt sensitive salt species are incompetent of providing low $Cl^-$ concentrations in chloroplasts under salinity by means of effective compartmentation between cytoplasm and vacuole (Seemann and Critchley, 1985). Hence, part of growth reduction owing to salinity in pepper may be associated with $Cl^-$ related decrease of leaf chlorophyll, which restricts net assimilation. Navarro et al., (2006) also reported negative effects of salinity on pepper growth, and they concluded that the yield reduction induced by salt stress can be linked to the toxic effects of $Cl^-$ accumulation in the plant tissues.

## Conclusion

Salt stress is one of the most widespread environmental hazards worldwide crop production, especially in arid and semi-arid regions, where land degradation, water deficit and population growth are already dominant concerns. Pepper is an important agricultural crop, because of its economic importance and the nutritional value of fruits (Navarro et al., 2006). It has been classified from moderately sensitive to sensitive under salinity conditions (Penella et al., 2017), even though cultivars with different salt tolerances have been reported in previous studies. In our research, significant differences for salt tolerance among mean of generations were observed in all the treatments. Leaf, shoot and root fresh and dry weights, total leaf area, total root length, and total root volume were generally reduced in different ratios under salinity stress. BC3-3 plants have significantly higher leaf and root fresh matter as compared to progenies of third backcrosses and their respective parents. The root dry matter varied from 0.86 g $plant^{-1}$ in BC3-3 to 0.25 g $plant^{-1}$ in Sena of 8 dS $m^{-1}$. 8 dS $m^{-1}$ NaCl decreased the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively than control plants. Up to now it was stated in many research that total leaf area formation is normally reduced when plants are under any case of stress. In our results, the significantly higher total leaf area formation was produced at the progenies of third backcrosses of BC3-3 plants. Salinity had a significant adverse effect not only on the plant growth and development, although also on leaf chlorophyll content of progenies of third backcrosses and their respective parents. Although this harmful effect was more obvious in respective parents than the progenies of third backcrosses. Leaf chlorophyll content are often well correlated in salt-stressed plants (Gong et al., 2013). Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Munns and Tester, 2008). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. Nonetheless, although ionic and water homeostasis are crucial parameters in abiotic stress tolerance, the maintenance of shoot vigor and leaf function are vitally important. The $Cl^-$ concentration in leaves increased with a higher NaCl concentration. In this way, Kopan accumulated high concentration of toxic ions ($Cl^-$) in plant tissues. Furthermore, regarding total root length, and total root volume, the significantly highest values were observed at the progenies of third backcrosses of BC3-3 plants. Summarizing, the

progenies of third backcrosses of BC3-3 was more tolerant to salinity than their respective parents (Kopan and Sena). This was highly associated with vigorous root growth (root fresh and dry weight, total root length and volume) and photosynthetically active leaves (total leaf area, leaf chlorophyll index, chloride exclusion) under hydroponic salt stress.

## Compliance with Ethical Standards

### Conflict of interest

The author declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

### Funding

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# The application of fish wastewater to improve the plant growth, development and yield of lettuce (*Lactuca sativa* L.)

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## Abstract

Demand for low-cost and affordable alternating sources of plant nutrient responses to boost the nutrient level of damaged arable farmlands has been a main concern for soil scientists, agronomists, and local farmers. The objective of this study is to investigate the effect of fish wastewater on the growth parameters, yield, and biomass productivity of lettuce (*Lactuca sativa* L.) as compared by using in aerated nutrient solution under deep water culture (DWC) technique. The experiment was carried out to investigate shoot and root fresh and dry weight, total leaf number, leaf chlorophyll content (SPAD), photosynthesis, leaf total chlorophyll (a+ b), leaf total carotenoid content, total leaf area, leaf NRA activity, total root length, root volume and average root diameter. Lettuce plants were examined by using an aerated deep-water culture (DWC) technique in a fully automated climate room for six weeks. The seedlings were transplanted onto 8 L continuously aerated pots containing mix of different ratios of fish effluent water with tap water with six different treatments (T1, T2, T3, T4, T5 and T6) and replicated three times. The fish wastewater effluents did not reduce the growth of lettuce plants. Shoot and root fresh and dry matter, total leaf number, leaf total chlorophyll (a+ b), leaf total carotenoid content, total leaf area, leaf NRA activity, total root length, root volume and average root diameter of lettuce plants were significantly increased with under T3 treatment (Tap water + 1.5 mM N + 50 ml Nutrient solution + 8 ml Fe + 1000 ml Fish effluent water). However, the lettuce plants grown under T4 treatment (Tap water + 1.5 mM N + 250 ml Fish effluent water) had the lowest shoot and root fresh matter, total leaf number, photosynthesis, total leaf area, leaf NRA activity, total root length, root volume and average root diameter. The compost derived from the fish wastewater plays an important role in supplying the nutrients for cultivating the lettuce plants. Also, in this study appreciable nutrients were significantly obtained in treatments treated with fish wastewater, as compared with the ground (tap) water. Thus, grown lettuce with aquaculture is a good source of nutrition for human consumption.

## Keywords

Fertilizer, Fish effluent water, *Lactuca sativa* L, Nutrient solution. Wastewater

## Introduction

Lettuce (*Lactuca sativa* L.) is one of the most popular leafy vegetables; grown around the world (FAO, 2018), it is considered to be a healthy source of minerals, fiber, vitamins, and antioxidant compounds (Baslam et al. 2011; Camejo et al. 2020). Several epidemiological studies have shown that the consumption of leafy vegetables such as lettuce is important for reducing the risk of chronic diseases, such as diabetes, cancer, and cardiovascular disease (Wang et al. 2011). These health benefits have been linked to a range of micro- and macro-nutrients, vitamins, and biological compounds, including carotenoids, anthocyanins, and phenolic compounds. Lettuce is generally grown under controlled environments, including hydroponic systems, greenhouse, and plant factories, with the quality of the product dependent on several factors such as light quality, nutrient composition, water level, and salt stress (Fu et al. 2012; Sofo et al. 2016; Camejo et al. 2020).

The world population is growing dramatically which is expected to increase from 7.7 billion in 2019 to 9.7

billion in 2050 (United Nations 2019). About 5–7 million ha (0.6%) of world cropland are lost annually due to population growth, land degradation, and urbanization (WWAP 2012). Population growth with loss of cropland has resulted in a gradual decline of cultivated land worldwide from 0.44 to 0.25 ha per person over the last 50 years. Finding enough food for this population involves increasing crop production with practical fertilizer application methods. Applying chemical fertilizers to the crop production has been used to increase crop yield, development and quality for decades. Though, present agricultural trends focus on searching for alternatives to chemical fertilizers because of environmental contamination, huge procurement costs and couples with improper application leading to soil quality degradation (Almamori and Abdul-Ratha, 2020). Additionally, the world demands quality food production, getting more yield and maintaining soil biodiversity most sustainably. Therefore, for the future, it is necessary to develop and adopt strategies that provide optimal nutrition for plants and improve crop yields, whilst at the same time minimizing environmental pollution (Ronga et al. 2015). The use of fish wastewater when applied to crops could be a better alternative method for efficient usage of limited water, reducing chemical fertilizer and pesticide use, enhancing crop yields, farm productivity and income. This is because of the accompanying nutrients in the fish wastewater, which could be beneficial for plant growth, yield and product quality. Previous studies have reported a significant increase in crop yields as well as an increased water use efficiency when crops are irrigated with fish effluents (Zajdband, 2011; Mariscal-Lagarda et al., 2012). Ramírez Sanchez et al. (2011) investigated the productivity of oregano in both aquaponics and hydroponics, and they reported higher fresh and dry yields in the case of aquaponics. Similarly, Hussain and Al-Jaloud (1995) and Limbu et al. (2017) reported a significant yield in barley and Chinese cabbage respectively the later, which yielded 80% more under fish effluent irrigation than the conventional production. Furthermore, Castro et al. (2006) stated an increase in tomato yield from 64.5 to 95.8 t/ha when the plants were irrigated with aquaculture and lower yield were recorded when the plants were irrigated with well (ground water). To the best of our knowledge, limited studies have investigated replacing chemical fertilizers in nutrient solution experiments with fish effluents in lettuce production (Dediu et al., 2012; Abbey and Anderson, 2019; Monsees et al., 2019; Huang et al., 2021). Therefore, the objective of this study is to investigate the effect of fish wastewater on the growth parameters, yield and biomass productivity of lettuce (*Lactuca sativa* L.) as compared by using in aerated nutrient solution under deep water culture (DWC) technique.

## Materials and Methods

### Experimental Set-Up

A hydroponic trial was set up using an aerated deep water culture (DWC) technique in a fully automated climate room in the Plant Physiology Laboratory of Erciyes University's Faculty of Agriculture, Department of Soil Science and Plant Nutrition, in Kayseri, Turkey. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 60-80%. The supplied photon flux in the growth chamber was almost 350 µmol $m^{-2}$ $S^{-1}$ with an intensity of 16/8 h (light/dark) photoperiod. The seeds of lettuce genotype (Bachus LOL9666 variety) of the plant materials were sown in multipots in a mixture of peat (pH: 6.0-6.5) and perlite in a 2:1 ratio. Plants were transferred to 8 L plastic containers after roots were washed from growth media, each pot was filled with nutrient solution and aerated by an air pump. Due to transplanting small seedlings, the solutions were changed completely in the first two weeks and subsequently every 7$^{th}$ day.

The trial was set up in a completely randomized block design (CRBD) with three replications and six plants in each replication. To prepare the nutrient solution for the hydroponic experiment, analytical grade (99% pure) chemicals with distilled water were used according to the Hoagland (modified) formulation. In the solution, 2000 µM nitrogen was supplied by using 75% calcium nitrate ($Ca(NO_3)_2$) and 25% ammonium sulfate ($(NH4)_2SO_4$) as the N sources. Moreover, the composition of the basic nutrient solution was as follows (µM): $CaSO_4$ (1000), $K_2SO_4$ (500), $MgSO_4$ (325), $KH_2PO_4$ (250), NaCl (50), $H_3BO_3$ (8.0), Fe-EDDHA (80), $ZnSO_4$ (0.4), $CuSO_4$ (0.4), $MnSO_4$ (0.4), $MoNa_2O_4$ (0.4). All the nutrients were replaced to prior concentrations when the N concentration in the solution fell from 2.0 mM to below 1.0 mM. Daily nitrogen concentration was checked by nitrate test strips (Merck, Darmstadt, Germany) with the aid of a NitracheckTM reflectometer. Distilled water was added every 2 days to replenish the water lost to evaporation, and the solution was changed weekly.

The fishpond was covered with a black net to reduce evapotranspiration losses. A fish a stage of fingerlings (mean weight (8-10 g)) was stocked into the pond in the laboratory condition. The pond was aerated with 1 hp ring blower that was connected with one airlift units to ensure efficient water aeration. The fish was fed 3-4 times to satiation daily with commercial pellets specific to each growth stage. The fish effluent water (3300 ml) was taken from the fishpond and transferred to 8-liter plastic containers. Then the tap water (4700 ml) was added into 8-liter plastic containers. Both of them were mixed and completed to 8 liters. Different levels (250 ml-500 ml-1000 ml) of fish water were taken from these containers and used in the experiment. The treatments used at the experiment were shown in Table 1.

### Plant Growth Measurements

Plant growth was measured by using three uniform plants from each replication. Shoot and root were fractioned into the leaf, stem and roots for the fresh weight determination. And then, samples were stored separately in paper bags and dried in a ventilated oven at 74 °C for 72 hours. Root to shoot ratio was calculated by dividing the root dry weight by the sum of leaf and stem dry weights.

Table 1. Treatments applied to lettuce grown hydroponically at the experiment

| Treatments (T) | |
|---|---|
| Treatment 1 (T1) | Tap water + 1.5 mM N + 200 ml Nutrient solution + 32 ml Fe + 250 ml Fish effluent water |
| Treatment 2 (T2) | Tap water + 1.5 mM N + 100 ml Nutrient solution + 16 ml Fe + 500 ml Fish effluent water |
| Treatment 3 (T3) | Tap water + 1.5 mM N + 50 ml Nutrient solution + 8 ml Fe + 1 L Fish effluent water |
| Treatment 4 (T4) | Tap water + 1.5 mM N + 250 ml Fish effluent water |
| Treatment 5 (T5) | Tap water + 1.5 mM N + 500 ml Fish effluent water |
| Treatment 6 (T6) | Tap water + 1.5 mM N + 1 L Fish effluent water |

### Total Leaf Number and Leaf Physiological Measurements

Each fully developed leaf was counted and recorded as a total leaf number (LN plant$^{-1}$). The total leaf area (cm$^2$) of the plants was measured with a leaf area measuring device LI-COR (LI-COR Model 3100, LI-COR. Inc., Lincoln, NE, USA). The measurements were carried out at the temperature of 25/22 °C (average day/night temperatures), the relative humidity of 60-80%. The supplied photon flux in the growth chamber was almost 350 µmol m$^{-2}$ S$^{-1}$ with an intensity of 16/8 h (light/dark) photoperiod. Prior to harvest, non-destructive measurements of the leaf-level $CO_2$ gas exchange (µmol $CO_2$ m$^{-2}$ s$^{-1}$) were done in a controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). Gas exchange in the leaves was performed on the youngest fully expanded leaves, using four replicate leaves per treatment in the third and fifth weeks of the growth period. The Minolta SPAD-502 chlorophyll meter was used to measure SPAD index. During the growth period, fully expanded leaves of whole plants for each treatment were twice measured for SPAD data.

### Leaf Total Chlorophyll (a+ b) and Carotenoid Content Measurements

A day before harvesting, extraction of the photosynthetic pigments from 100 mg (0.1 g) of fresh leaf samples from each replication of the two treatments was taken for measuring the leaf total chlorophyll and carotenoid contents using UV-VIS Spectroscopy. The leaf samples used for chlorophyll and carotenoid determinations were of the same physiological age as those used for the leaf net photosynthesis measurements. The samples were put into 15 ml capped containers where 10 ml of ethylene alcohol of 95% concentration was added. They were then kept in darkness at room temperature overnight, to allow the extraction of the leaf pigments. Measurements were done using the spectrometer (UV/VS T80+ of PG Instruments Limited, UK) at wavelengths of 470 nm, 648.6 nm, and 664.2 nm. Total chlorophyll (Total-Chlo) and total carotenoids (TC) were then estimated from the spectrometric readings using the formulae of Lichtenthaler (1987).

Total-Chlo (mg/g plant sample) = [(5.24 WL664.2-22.24 WL648.6 x 8.1]/ weight of plant sample (g)

TC (mg/g plant sample) =[(4.785 WL470 + 3.657 WL664.2) -12.76 WL648.6) x 8.1]/ weight of plant sample (g)

Note: WL470, WL648.6 and WL664.2 refers to spectrometric readings at wavelength 470 nm, 648.6 nm and 664.2 nm respectively.

### Root Morphological Measurements

The plant root morphological parameters such as total root length (m), total root volume (cm$^3$) and average root diameter (mm) were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc., Québec, QC G1V 1V4, Canada) in combination with recording device of Epson Expression 11000XL scanner (Long Beach, CA, USA).

### Leaf Nitrate Reductase (NRA) Activity Measurement

Nitrate reductase (NRA) activity in the leaf was determined following the method proposed by Harley (1993). At harvesting fresh plant samples were taken and chopped into pieces; two grams of the latter were placed in each of two falcon tubes and labeled time-0 (T0) and time-60 (T60). The tubes were covered with aluminum foil to be screened from light. Ten ml of assay buffer solution [100 mM phosphate buffer, pH 7.5; 30 mM $KNO_3$; 5% (v/v) propanol] was added to each tube (T0 and T60). The T0 container was immediately placed into boiling water for five minutes, removed and allowed to cool to room temperature. While the T60 was kept for 60 minutes at room temperature; after which it was also placed into boiling water for five minutes and allowed to cool to room temperature. To detect nitrite in the assay tubes, the optical density (OD) of each standard tube was determined at 540 nm wavelength in the spectrometer.

### Statistical Analysis

All measured physiological and morphological parameters were analyzed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of genotypes and salt and their interactions on the variables analyzed. Levels of significance are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns means not significant. Differences between the treatments were compared using Duncan's Multiple Test ($p < 0.05$).

## Results and Discussion

### Biomass Production and Partitioning

The results of lettuce harvest after 35 days of cultivation are shown in Table 2. The shoot and root fresh matter, and total leaf number were significantly ($p < 0.001$) affected by the treatments. It was obvious that treatment T3 performed remarkably better than other treatments. The significantly highest shoot (265.5 g plant$^{-1}$) and root fresh matter (48.7 g plant$^{-1}$), and total leaf number (60 LN plant$^{-1}$) were produced in the treatment T3. Though, significantly lowest shoot (51.1 g plant$^{-1}$) and root fresh matter (10.9 g plant$^{-1}$), and total

leaf number (47 LN plant$^{-1}$) were produced in the treatment T4 (Table 2). This result shows that in the treatment T3 of lettuce is more nutritious than others which helps lettuce to grow and develop better. While the treatment T4 is less nutritious so it grows and develops slowly. The average fresh weight of lettuce in this study was greater than fresh lettuce weights from other aquaponic studies conducted (Table 2). The average fresh weight of lettuce (Bachus LOL9666 variety) from this study (152 g) was more than the lettuce genotype of 'Salanova' from both aquaponic (89 g) and hydroponic production (91.18 g; Søberg, 2016). According to the University of California Davis, the average fresh weight of a loose-leaf lettuce in field production is 415.79 g (Takele, 1996). This is significantly more than the average fresh weight of any cultivar from this study or other aquaponic and hydroponic lettuce production studies reviewed. It appears that across studies, the average weight of lettuce produced in an aquaponic or hydroponic production system will be less than half of typical field production.

Al-Jaloud and Hussein (1995) stated that increase in the yield of wheat and French bean with the application of fish pond, while Nadafi et al. (2005) wood also reported increased growth rate and improved quality of garden purslan, sweet basil, and radish and cucumber crops with fish pond water application. Also, higher yield of fresh bean pod and fresh Kale leaf increases via fish pond water application as a source of fertilizer (Wood et al. 2001). These results showed that irrigating with fish pond water; results in greater yield. This also revealed that nutritional values are present in the fish pond water which is the obvious factors responsible for the improved yield increased.

Delaide et al., (2016) reported that the supplementation of fish water with mineral fertilizer increased the fresh weight of lettuce by nearly 40%. In contrast, Suhl et al. (2016, 2018) documented no significant differences in tomato yield between conventional hydroponics and supplemented aquaponics.

Table 2. Shoot and root fresh weight and total leaf number of lettuce grown under different six treatments

| Treatments (T) | Shoot Fresh Weight (g plant$^{-1}$) | Root Fresh Weight (g plant$^{-1}$) | Total Leaf Number (LN plant$^{-1}$) |
|---|---|---|---|
| T1 | 215.6 c | 24.1 c | 58 c |
| T2 | 220.5 b | 17.3 d | 59 b |
| T3 | 265.5 a | 48.7 a | 60 a |
| T4 | 51.1 f | 10.9 f | 49 e |
| T5 | 55.1 e | 13.9 e | 47 f |
| T6 | 106.6 d | 35.4 b | 52 d |
| F-Test: | | | |
| Treatments | *** | *** | *** |

[1]Values denoted by different letters are significantly different between treatments within columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 3 shows the shoot and root dry weight and root: shoot ratio of lettuce plants grown in different treatments hydroponically. The shoot and root dry matter, and root:shoot ratio were significantly ($p < 0.001$) affected by different treatments. The results show that shoot (17.6 g plant$^{-1}$) and root dry weight (2.5 g plant$^{-1}$) were significantly higher at the treatment of T3. The lowest shoot dry matter of plants is at treatment T5, and the average value is 4.8 g plant$^{-1}$. The highest root:shoot ratio of lettuce plants was observed at the treatment T5 recording an average value of 0.23 g g$^{-1}$. When comparing the shoot dry matter among the treatments, the results show that using the fish effluent water (1000 ml) combined with nutrient solution (50 ml) has helped to increase the shoot dry matter faster compared with using fish effluent water only. This is an indication that there is an obvious relationship between the shoot and root dry weights with respect to volume of fish pond water applied. Similar results were observed by Akindele et al. (2021) at sweet pepper. They stated that the highest number of leaves, stem girth, biomass and sweet pepper yield, root weight and leaf area index (LAI) were recorded in treatment, 100% of Potential Evapotranspiration (PET) for aquaculture water (T2) as compared to 50% of PET for aquaculture water (T1) and 100% of PET for ground water (T0). Li et al (2021) stated that the rice and fish yield increased in integrated system compared to monoculture. Similar results were obtained by García-Santiago et al (2021). They stated that leaf dry weight, total plant biomass dry weight, fruit number and total yield were higher in the organic fertilization treatment (including fish-derived protein hydrolysate as an N-source), surpassing the conventional treatment by 35%, 9%, 21%, and 4% for these parameters, respectively, though the difference was only significant for leaf dry weight in grape tomatoes.

### Leaf Chlorophyll Content (SPAD), Photosynthesis, Leaf Total Chlorophyll (a+ b) and Carotenoid Content, Total Leaf Area and Leaf NRA Activity

The results indicate that leaf chlorophyll content (SPAD), photosynthesis, leaf total chlorophyll (a+ b), leaf total carotenoid content, total leaf area and leaf NRA activity at the end of the growing cycle were significantly ($p < 0.001$) affected by treatments (Figure 1). Concerning SPAD, lettuce plants grown under the treatment T4 had the highest leaf chlorophyll content, reaching an average of 34.2 SPAD as shown in Figure 1A. This was closely followed by lettuce plants grown

under T3 treatment, with an average number of 31.5 SPAD. The treatment T5, however, recorded the significantly lowest with 20.8 SPAD. In plants, chlorophyll is a green pigment, which is vital as far as photosynthesis is concern; it helps in transforming light energy to chemical energy during the activities of photosynthesis. Also, the amount of chlorophyll present in a leaf is very paramount to depicts the growth of plants (Bannari et al. 2007). Therefore, in crop production chlorophyll is necessary for photosynthetic activities.

Table 3. Shoot and root dry weight and root:shoot ratio of lettuce grown under different six treatments

| Treatments (T) | Shoot Dry Weight (g plant$^{-1}$) | Root Dry Weight (g plant$^{-1}$) | Root: Shoot Ratio (g g$^{-1}$) |
|---|---|---|---|
| T1 | 13.5 b | 1.4 c | 0.10 e |
| T2 | 13.4 c | 1.1 d | 0.08 f |
| T3 | 17.6 a | 2.5 a | 0.14 d |
| T4 | 5.4 e | 1.1 d | 0.20 b |
| T5 | 4.8 f | 1.1 d | 0.23 a |
| T6 | 11.9 d | 2.2 b | 0.18 c |
| F-Test: | | | |
| Treatments | *** | *** | *** |

[1]Values denoted by different letters are significantly different between treatments within columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Regarding photosynthesis, the results showed that treatment T1 performed best compared to the rest of the treatments. It recorded an average of 8.16 µmol $CO_2$ $m^2$ $s^{-1}$ photosynthesis as shown in Fig. 1B. This was followed by treatment T2 with the value of 5.82 $CO_2$ $m^2$ $s^{-1}$ and closely by treatment T3 with the value of 5.61 $CO_2$ $m^2$ $s^{-1}$. Data in Fig. 1C and 1D shows that lettuce plants grown mixed treatment with 1000 ml fish effluents and 50 ml nutrient solution (T3) obtained significantly higher values in leaf total chlorophyll (a+ b) and leaf total carotenoid content at $p < 0.001$, with a value of 1.269 µmol $g^{-1}$ and 0.214 µmol $g^{-1}$, followed by the mixed treatment with 500 ml fish effluents and 100 ml nutrient solution (T2) treatment at 0.752 µmol $g^{-1}$ and 0.133 µmol $g^{-1}$ for leaf total chlorophyll (a+ b) and leaf total carotenoid content respectively. Results from treatment T4 had significantly lower leaf total chlorophyll (a+ b) and leaf total carotenoid content with a value of 0.451 µmol $g^{-1}$ and 0.083 µmol $g^{-1}$. Similar results were observed by Akindele et al. (2021) at sweet pepper. They stated that the highest number of leaves, stem girth, biomass and sweet pepper yield, root weight and leaf area index (LAI) were recorded in treatment, 100% of Potential Evapotranspiration (PET) for aquaculture water (T2) as compared to 50% of PET for aquaculture water (T1) and 100% of PET for ground water (T0). Few studies have attempted to clarify the effect of fish effluents in crop production but mostly under the rice-fish culture (Jamu and Piedrahita, 2002; Koide et al., 2015). A few researchers have examined the influence of organic fertilization on marjoram crops and stated paramount positive effects (Gharib et al., 2008; Naguib, 2011).

In terms of total leaf area, the treatment T3 gave the highest value of 4331.1 $cm^2$ $plant^{-1}$, while followed by the treatment T1 with the value of 3347.2 $cm^2$ $plant^{-1}$, respectively. Evidently, the treatment T4 gave the lowest value (1363.7 cm2 $plant^{-1}$) for the total leaf area production. Thus, affirmed that grown plants with fish pond water (1000 ml) does directly increased leaf area development in lettuce production. This is as a result of enhancement of photosynthesis due to higher total leaf area (Ogbonnaya et al. 1998).

Results of leaf NR activity indicates that plants grown under treatment T3 recorded the highest leaf NR activity, though plants grown under treatment T4 recorded the lowest leaf NR activity. Nitrogen is a decisive nutrient for plant growth (Hawkesford et al., 2012). Therefore, the focus for mixing the nutrient solution in the present study was to align N in the different treatments, which was only possible with regard to the total N.

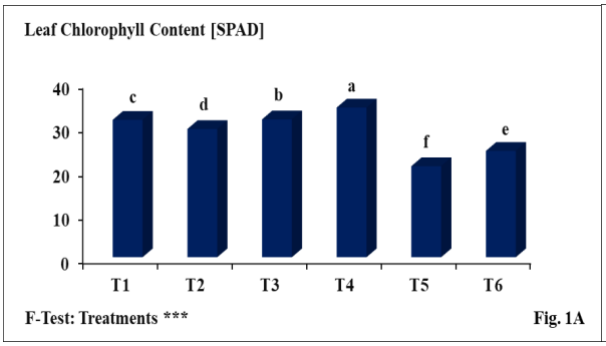

Fig. 1A

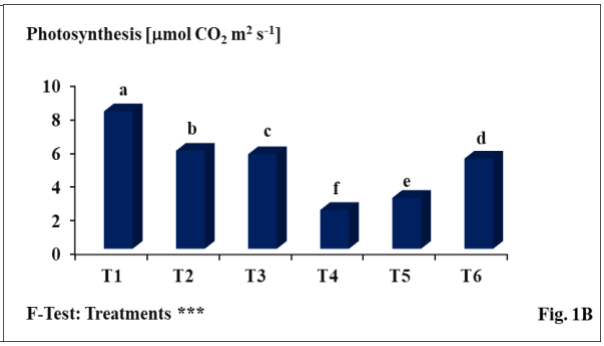

Fig. 1B

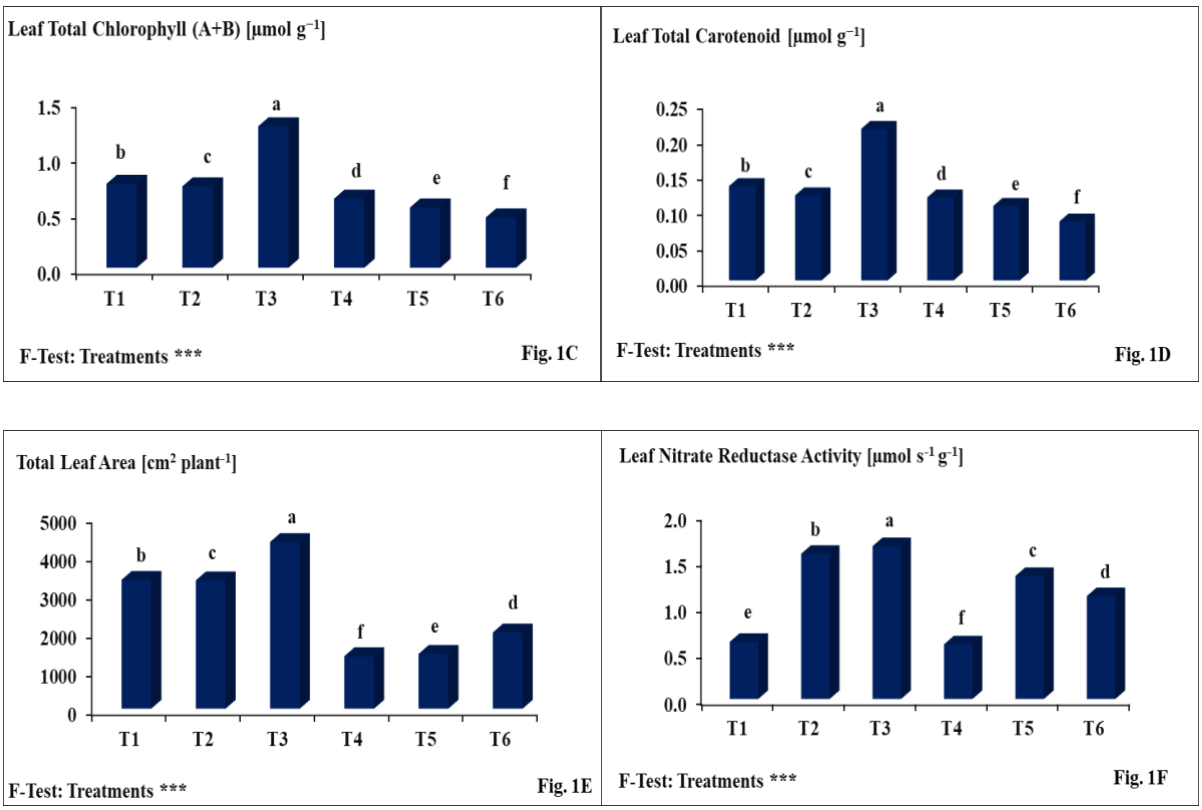


Figure 1. Leaf chlorophyll content (SPAD) (A), photosynthesis (B), leaf total chlorophyll (a+ b) (C), leaf total carotenoid content (D), total leaf area (E) and leaf NRA activity (F) of lettuce grown under different six treatments. [1]Values denoted by different letters are significantly different between treatments within columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

## Total Root Length, Total Root Volume and Average Root Diameter

Total root length, root volume and average root diameter were significantly ($p < 0.001$) affected by different treatments (Table 4). Results show that plants grown at the treatment T3 recorded relatively higher values for total root length (476.8 m plant$^{-1}$), root volume (45.4 cm$^3$ plant$^{-1}$) and average root diameter (16.12 mm) compared to the other treatments. This was closely followed by the treatment T6. This result shows that in the treatment T3 of lettuce is more nutritious than other treatments which helps lettuce to grow and more root development better. Similar results were observed by Akindele et al. (2021) at sweet pepper. They stated that the highest number of leaves, stem girth, biomass and sweet pepper yield, root weight and leaf area index (LAI) were recorded in treatment, 100% of Potential Evapotranspiration (PET) for aquaculture water (T2) as compared to 50% of PET for aquaculture water (T1) and 100% of PET for ground water (T0).

Table 4. Total root length, root volume and average root diameter of lettuce grown under different six treatments

| Treatments (T) | Total Root Length (m plant$^{-1}$) | Total Root Volume (cm$^3$ plant$^{-1}$) | Av. Root Diameter (mm) |
|---|---|---|---|
| T1 | 304.5 c | 23.6 c | 7.63 c |
| T2 | 303.6 d | 19.1 d | 4.99 d |
| T3 | 476.8 a | 45.4 a | 16.12 a |
| T4 | 125.5 f | 12.8 f | 3.91 f |
| T5 | 171.0 e | 14.6 e | 4.55 e |
| T6 | 420.8 b | 33.3 b | 11.07 b |
| F-Test: | | | |
| Treatments | *** | *** | *** |

[1]Values denoted by different letters are significantly different between treatments within columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

## Conclusion

This study was conducted to investigate the effect of fish wastewater on the growth parameters, yield and biomass productivity of lettuce (*Lactuca sativa* L.) as compared by using in aerated nutrient solution under deep water culture (DWC) technique. Results showed that the lettuce plants grown under T3 treatment (Tap water + 1.5 mM N + 50 ml Nutrient solution + 8 ml Fe + 1000 ml Fish effluent water) produced the highest

shoot and root fresh and dry matter, total leaf number, leaf total chlorophyll (a+ b), leaf total carotenoid content, total leaf area, leaf NRA activity, total root length, root volume and average root diameter, while the lettuce plants grown under T4 treatment (Tap water + 1.5 mM N + 250 ml Fish effluent water) had the lowest shoot and root fresh matter, total leaf number, photosynthesis, total leaf area, leaf NRA activity, total root length, root volume and average root diameter. The compost derived from the fish wastewater plays an important role in supplying the nutrients for cultivating the lettuce plants. Also, in this study appreciable nutrients were significantly obtained in treatments treated with fish wastewater, as compared with the ground (tap) water. Thus, grown lettuce with aquaculture is a good source of nutrition for human consumption.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Application of swat hydrological model to assess the impacts of land use change on sediment loads

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## Abstract

Controlling and reducing the watershed's erosion and sedimentation is essential to ensure the continuity of projects implemented to develop land and water resources and improve sustainability, performance, and longevity. Sediment control is also critical in managing the river basin in limiting the transport of solids, improving water quality, sustaining aquatic life, and preventing damage to downstream aquatic environments and ecosystems. Estimating the potential effects of land-use changes on surface runoff and soil erosion requires distributed hydrological modeling methods. In addition to naturally occurring sediments, changes in land-use types for different applications can be a primary cause for the increase in sediment rates in the watershed. This study used the Soil and Water Assessment Tool (SWAT), a rainfall-runoff model, to evaluate land use/cover changes (i.e., deforestation) and their impact on sediment load under different scenarios. For the baseline (no changes) scenario, the watershed is calibrated using the flow and sediment data measured from the rain gauge station during the time step to estimate the post-deforestation changes at the sub-catchment scale of the study area. The study results indicated that the total surface runoff and sediment yield for the selected sub-catchment in the deforestation scenario with the highest spatial distribution, due to the high erosivity (24% increase) of excessive surface runoff after deforestation, sediment yield increased 3.5-fold. In contrast, due to the removal of trees and vegetation's canopy, the evapotranspiration, leaf area index, and dissolved oxygen transported into reach showed the inverse ratios, and the values decreased by 5%, 24, and 17%, respectively, in compared with the baseline scenario. In terms of watershed management, therefore, the application of hydrological models such as SWAT rainfall-runoff and erosion models can be a helpful method for decision-makers to apply for the protection of forests from intensive impacts such as deforestation and limiting their socio-environmental effects.

## Keywords

Rainfall-runoff model, Deforestation, Agriculture, Soil erosion, Watershed management, Göksu-Çanakdere

## Introduction

Erosion and sedimentation issues and their impact on water resources and the environment are unavoidable and remain significant challenges for watershed management with high soil erosion rates. Erosion processes remove rock or soil and lead to the formation of mineral sediment. The sediment moves with water (surface runoff), wind, or ice, and deposits in new locations by gravity acting on the particles. Water's kinetic energy causes the wash of sediments down from the surface into a stream and eventually to the river's delta. There are complex interactions on the scale of river sections and basins, which affect the movement of sediments and the generation of habitat types in the river (Bettess et al., 2011; Gellis et al., 2016). Sediment can accumulate under, in, or around structures such as bridges, conduits, reservoirs, and water intakes. This process leads to cracks in the riverbed, severe stream instability, and eventually unusual flow around engineering structures such as bridge piers or culverts. Unlike frequent low-flow flood events, rare high-flow flood events can transport more sediment, leading to

sudden sediment accumulation. Different phenomena such as ground deformations or landslides can also lead to the overrun of sediments in the river system and form an unexpected build-up of sediments deposition.

In addition to the naturally-occurring sediment, it may also originate from human practices on the land. The impact of agriculture, deforestation, mining, urbanization, and road building on soil erosion rates is inevitable (Amundson et al., 2015; Hassan et al., 2017). In agricultural activities, soil erosion occurs through the field's topsoil wearing away off by the natural physical or chemical forces of water and wind or through forces incorporated with the farming activities such as plowing and tillage (Ritter, 2012). The world's rapid population growth leads the urbanization and changing land-use. On the other hand, population growth leads to increased food demand in many countries. Hence the agricultural sector is being put under excessive pressure to produce and provide enough food for the growing communities. With soil erosion, the most productive and valuable soil profile used for agricultural purposes will be lost through the reduced capacity of the soil for storing water and nutrients with the poor physical and chemical properties of the subsoil (Langdale et al., 1992). Degraded land is also often unable to retain water, exacerbating flooding. The impact of soil erosion is not just the loss of fertile land. It leads to increased water pollution and reduces dissolved oxygen, reducing fish and other species. Therefore, the loss of productive topsoil with rich organic matter lowers the crop yield potential due to the degraded soil structure and the reduction of nutrients that were contained in the organic matter (Brevik, 2006; Wardle et al., 2004), increased production costs (Pimentel and Burgess, 2013), and thus will cause the increased demand for agricultural commodities.

Consequently, the above reasons will create an impetus to convert forests and grasslands into farmland (Izquierdo and Grau, 2009). Forests have a variety of ecological and hydrological benefits, for example, contributing to enhanced infiltration and water retention. Therefore, filtration in the soil column helps to improve water quality (Bredemeier, 2010). Furthermore, the combining effects of vegetation, litter cover, and less water yield imply lower erosion rates under forest cover, primitive sedimentation, mitigating flood hazards, and affecting both the soil quality and the downriver aquatic environments ( Farley et al., 2005; Owens et al., 2005; Schüler, 2006).

Degradation of forests for agriculture and timber leads to a significant increase in soil erosion rates by losing forest-derived leaf litter and plant roots. The role of the leaf litter is to protect the soil below from wind and water erosion and protect the plant roots that hold soil particles to and below the soil surface. (Avwunudiogba and Hudson 2014; Pimentel et al., 1995; Wenger et al., 2018). In addition to logging, forest fires have been the most common method of destroying forests. Forest fires either have a natural origin or are caused by human activities. Depending on the situation, natural wildfires are caused mainly by lightning, volcanoes, meteors, and coal seam fires. However, on a global scale, only about 4% of forest fires are caused by natural causes; in all other cases, humans are responsible for fires-whether directly or indirectly, intentionally or due to negligence (WWF, 2017). The anthropic causes of fires are agriculture displaying and ranching, which are constitute the highest level of association with forest fires (Juárez-Orozco et al., 2017).

Forest degradation by converting forest lands to dry farming and deforestation-induced impacts soil redistribution. In addition, these activities can lead to species loss and degradation of ecosystem functions, consequently affecting terrestrial ecosystems (Maxwell et al., 2016; Rocha-Santos et al., 2016). Therefore, through the changes in hydrological processes, degradation of forests for agriculture cause clear impacts to the freshwater in the downstream and estuarine environments with an increase in the amount of sediment (Iwata et al., 2003; Jenkins et al., 2007; Pattanayak and Wendland, 2007).

Predicting the potential effects of land-use changes, for example, deforestation and degradation of forests for agriculture on runoff and soil erosion, require the development of distributed hydrological modeling methods. The ecological, biological, climatological, biodiversity, socio-economic, and hydrological impacts of the deforestation and degradation of the forests have been investigated throughout various studies (Aliye et al., 2014; Bonan, 1999; Chrisphine et al., 2015; Hughes et al., 2000; Li et al., 2016; Symes et al. 2018; Wilk et al., 2001). Conversely, the potential of applications of the hydrologic and numerical models have not been thoroughly investigated to improve the conception of deforestation impacts on runoff and erosion-sedimentation and to plan relevant mitigation strategies by managers against the negative consequences of degradation of the forest,

This study investigates the potential of applying the SWAT hydrological model to examine the impact of degradation of forests for agriculture on sediment load. The purpose of this study is to evaluate the hydrological consequences of possible deforestations and their effects on streamflow and use runoff and erosion models to analyze their respective impacts on sediment load.

## Materials and Methods

In the context of this work, we investigated the effect of land-use changes, namely the degradation of forests for agricultural activities, on surface runoff and sediment yield at the sub-basin scale. To this end, we designed land-use change (deforestation) scenarios. To predict the hydrological response to the changes, we applied the rainfall-runoff model using the meteorological and basin characteristic data (land-use, soil, and topography). The model is calibrated using observed flow and suspended sediment concentration data. In terms of management practices, the findings in this study can be used in dealing with hydrological risks and environmental issues in the Göksu-Çanakdere rivers' watershed.

### Site description

The Göksu-Çanakdere rivers' watershed (40° 59” N, 29° 51” E), with an area of approximately 800 km$^2$, is in north-east of Istanbul and west of Kocaeli province, Turkey, reaching into the Black Sea (Figure 1). Its climate is characterized by the Black Sea region, the Balkans, and Anatolia. The two main tributaries in the study area are Göksu and Çanakdere, with a total length

of 122 and 77 km, respectively. The geological types include clay-limestone, sandy limestone, sandstone, and limestone (Citiroglu et al., 2011). The topographic pattern is partly undulating, ranging from 1 to 649 m elevation. The annual temperatures range from 8°C to 15°C, and annual rainfall ranges from 350 to 1400 mm (Aksoy et al., 2010). The average annual rainfall in the basin is about 815 mm, and the average annual temperature is about 14.8 °C. (State Meteorological Service (MGM). Flow and suspended sediment data were purchased from the Turkish State of Hydraulics (DSI), which was collected from rain gauge stations located in major rivers. Göksu-D02A004 (41° 04' 40" N, 29° 46' 34" E), and Çanakdere-D02A149 (41° 04' 19" N, 29° 51' 08" E) are two rainfall stations used to measure flow and sediment data. The total rainfall catchment area for collecting data from these two stations is 782 square kilometers. The predominant land-use type in the watershed is agriculture (55.2%) and forest (43.6%). The largest forest area is in the central part of the watershed between Istanbul and Kocaeli Province, mainly covering Pure Oak Stands forests, with the rest being Mixed Stands Forest species (Atalay, 1986).

### Land-use change scenario

Forests after agricultural lands are the most extensive in the region. Since most of the watershed is dedicated to agricultural activities and the fast-growing need for agricultural products to provide food resources, thus deforestation and conversion of forests into agricultural land is possible in the study area. Forest fires have always been the most common method of destroying forests and turning forests into farmland. Inland management agricultural activities play an important role in erosion control, agricultural pollutants, and water quality. Changes in land-use for agricultural purposes will also affect the hydrological response of the watershed. To study the impact of deforestation (i.e., after different forest fire scenarios) on the watershed, such as surface runoff, erosion, and sediment load, we designed baseline (pre-deforestation) and post-deforestation scenarios. This study was performed in two phases. The first phase corresponds to the baseline scenario. The model is calibrated and verified based on current land-use and meteorological data that have not changed from 2005 to 2017. The relevant rainfall event characteristics and model parameters did not change for the first scenario. In the second phase of the study, the forest patches which are destroyed due to the forest fires (forest fire scenarios) and converted to the non-irrigated arable land are defined for use in the model to analyze the effects of land-use change and its impacts on flow rates and sediment fluxes. In the proposed scenarios, homogeneous forest patches with 25%, 50%, and 100% burnt forest areas are defined for use in the model (Table 1). In this step, the land-use map is modified and used as a model input in the post-deforestation scenarios.

### SWAT Model

Hydrological modeling involves simulating the conversion process of precipitation to runoff. In general, the hydrological model has two main components. In the first component, precipitation contributes to the formation of the hydrograph, while in the second component, the water flow distribution is used to determine the hydrograph profile. Therefore, the rainfall-runoff process can include rainfall simulation in the basin, infiltration and rainwater loss, and finally, the trend and movement of excess water in the area. In contrast, the first three processes are related to the first component and the last process attributed to the second component of the hydrologic model. This study has applied the SWAT model, a physical-conceptual model, to simulate the hydrological conditions and sediment response for the current watershed situation and different forest fire scenarios. The SWAT hydrological model is a semi-distributed model functioning on daily or sub-daily time steps and process-based, time-continuous simulation. It can be implemented for different management conditions over extended periods in small to large complex watersheds divided into hydrological response units (HRUs) based on dem, soil, and land-use types. The model developed to estimate the impacts of different management methods on hydrological and water quality processes, sediment yield, and pollution loads (Arnold et al., 1993; Yen et al., 2014) and is also used to analyze the effects of climate, soil, vegetation and agricultural activities on the discharge and chemical yields in watersheds. SWAT is being applied to model streamflow, sediment, and nutrient transport within a watershed (Neitsch et al., 2011) and find a good action plan, which depends mainly on the integrated watershed model (Collins and McGonigle, 2008; De et al., 2013). This model anticipates the effects of land management practices or climate change on sediment transport and hydrological response at the surface over the water catchments with different soils, land uses, and management practices (Neitsch et al., 2005).

### Model set-up

In the SWAT model, the watershed is subdivided into multiple micro-basins, and each micro-basin is further subdivided into homogeneous parts named hydrological response units (HRUs). Each HRU is a homogeneous combination of land-use, management, topographical, and soil characteristics in each sub-basin (Arnold et al., 2012) to calculate water balance. Water balance in each HRU is an essential issue behind all processes in SWAT, as it affects plant growth, sediments movement, nutrients, pesticides, and pathogens. Water balance (Equation 1) is a function of inputs driven by climate parameters such as rainfall, temperature (maximum and minimum), solar radiation, wind speed, and relative humidity. The hydrological processes simulated by SWAT are including of surface runoff, evapotranspiration, lateral flow, infiltration, canopy storage, percolation, groundwater flow, tile drainage, water redistribution in the soil profile, return flow and recharge by infiltration from surface waters, ponds, and tributaries.

$$SW_t = SW_0 + \sum_{i=1}^{t}\left(R_{day} - Q_{surf} - E_a - w_{seep} - Q_{gw}\right) \quad (1)$$

where, the final soil water content, $SW_t$(mm $H_2O$) at the simulation time $t$ (days), is obtained from the sum of $SW_0$ (mm $H_2O$), the initial soil water content on day $i$, and the amount of total precipitation, $R_{day}$ (mm $H_2O$) on day $i$, when the total losses on day $i$ including the amount of surface runoff, $Q_{surf}$(mm $H_2O$), the amount of evapotranspiration, $E_a$(mm $H_2O$), the amount of water that percolates from the soil profile, $w_{seep}$(mm

$H_2O$), and the amount of return flow $Q_{gw}$(mm $H_2O$), are subtracted from the total precipitation. SWAT uses the SCS curve number procedure (USDA, 1972) and the Green and Ampt (Green and Ampt, 1911) infiltration method to predict surface runoff. Penman-Monteith (Monteith, 1965), Hargreaves (Hargreaves and Samani, 1985), and Priestley-Taylor (Priestley and Taylor, 1972) are three methods recommended by the model to estimate potential evapotranspiration (PET). In order to predict the erosion of each HRU and the sediment yield of the sub-basin, the SWAT model uses the Modified Universal Soil Loss Equation (MUSLE) (Williams, 1975), as shown in Equation 2.

$$Sed = 11.8 * \left(Q_{surf} * q_{peak} * area_{hru}\right)^{0.56} * K_{USLE} * C_{USLE} * P_{USLE} * LS_{USLE} * CFRG \quad (2)$$

In which, $Sed$ is the sediment yield to the stream network at the outlet on a given day (metric tons), $Q_{surf}$ is the surface runoff volume from a given rainfall event (mm $ha^{-1}$), $q_{peak}$ is the peak flow rate in $m^3 s^{-1}$, $area_{hru}$ is the area of the HRU (ha), $K_{USLE}$ is soil erodibility factor (0.013 metric ton $m^2$ h-/($m^3$ – metric ton cm)) which is a soil property available from the Universal Soil Loss Equation (USLE), $C_{USLE}$ is the USLE cover (crop/vegetation) management factor and can be derived from land cover data, $P_{USLE}$ is the USLE support conservation practice factor, the erosion baseline practice factor, which is a field-specific value, $LS_{USLE}$ is the USLE topographic (slope length-gradient) factor, and $CFRG$ is the coarse fragment factor.

SWAT requires different input data to represent water and sediment yield spatial variability. The required input data are soil and land use/cover feature maps, topographic maps (Digital Elevation Model (DEM)), and climate data. The watershed's land-use/cover types are mainly agricultural and forest land. The CORINE Land Cover (CLC, 2018) dataset of the EU Copernicus Land Monitoring Service is used as land use/cover data to generate hydrological response units (HRU) for each sub-basin area. A significant land use/land cover is divided into 16 land-use categories, converted from the original land-use category to the appropriate SWAT land-use classification code, and defined using a lookup table, as shown in Table 2. The soil characteristic data was obtained from the Turkish soil dataset prepared by an interdisciplinary team composed of soil scientists, geologists, geomorphologists, and GIS experts (Cullu et al., 2018). The main soil types were reclassified according to the soil texture and hydrological soil groups (FAO soil classification), provided by the European Commission and the European Soil Bureau Network (Kük and Burgess, 2010). The meteorological data is obtained from the MGM (State Meteorological Service) for thirteen hydrological years (2005 to 2017) and simulated the SWAT model. The measured flow and sediment data collected from the Göksu and Çanakdere rain gauge stations are used for model calibration and verification. The main steps of the input and fitting process of the SWAT model are shown in Figure 2.

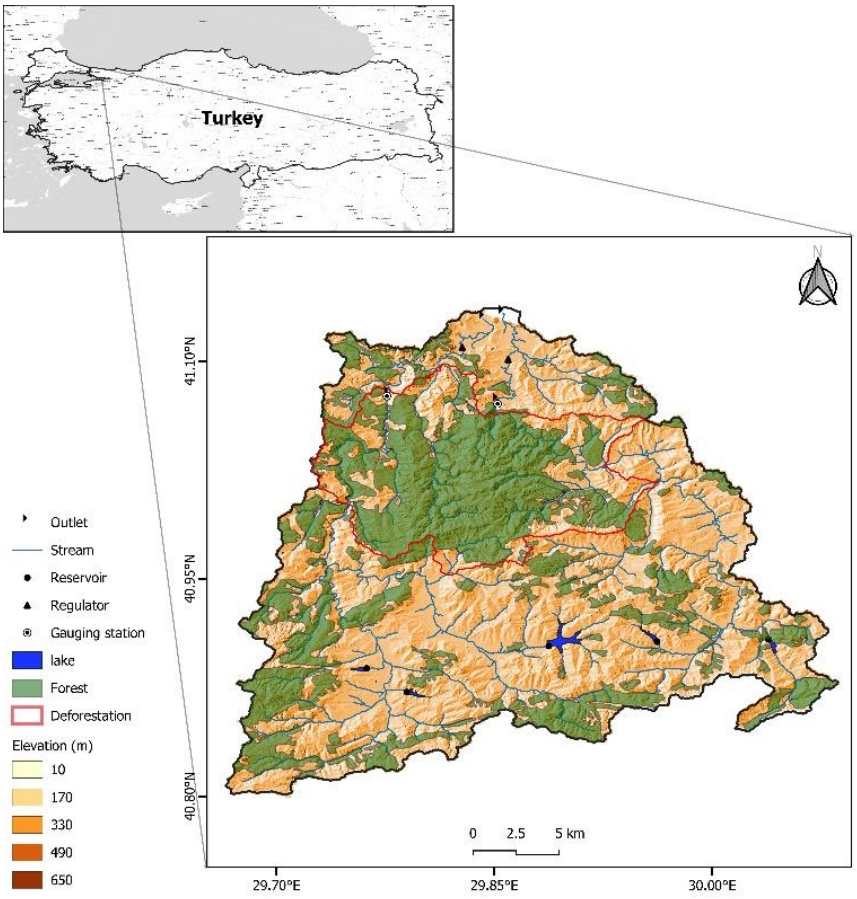


Figure 1. The location map of the study area, Göksu-Çanakdere rivers' watershed

Table 1. Description of scenarios used in this study. The mean degraded area is relative to the total watershed area (%)

| Scenarios | Forest Patches Condition | Land-use Change (Forest Fires) (%) | Degraded Forest Area (ha) | Mean Degraded Area to Total Watershed Area (%) |
|---|---|---|---|---|
| Baseline | No land-use change | - | - | - |
| Scenario 1 | Pastures + woodland + Mixed forest | 25 | 2900 | 3.5 |
| Scenario 2 | Mixed forest + Broad leaved forest | 50 | 5800 | 7 |
| Scenario 3 | Broad leaved forest | 100 | 11000 | 14 |

## Model performance evaluation

The model evaluation was performed based on the calibration and validation with a comparison of the measured and simulated discharge and sediment loads to constrain the model and achieve more robust characterizations of the land and the stream phases over the time step. The coefficient of determination (R2) and the Nash–Sutcliffe efficiency coefficient (NSE) (Nash and Sutcliffe, 1970) are two objective functions based on error statistics that were used as statistical and graphical model evaluation techniques to assess the model performance (Moriasi et al., 2007). The values of $R^2$ and NSE are determined by Equations 3 and 4, respectively.

$$R^2 = \frac{[\sum_i (Q_{m,i} - \bar{Q}_m)(Q_{s,i} - \bar{Q}_s)]^2}{\sum_i (Q_{m,i} - \bar{Q}_m)^2 \sum_i (Q_{s,i} - \bar{Q}_s)^2} \quad (3)$$

$$NSE = 1 - \frac{\sum_i (Q_m - Q_s)^2}{\sum_i (Q_{m,i} - \bar{Q}_m)^2} \quad (4)$$

where $Q$ is a variable (for example, discharge), $m$ and $s$ represent observed or simulated, and $i$ is the $i^{th}$ observed or simulated data.

Table 2. Land use/cover distribution of the study area based on CORINE land cover classes classification and the converted land cover classes for the corresponding SWAT land cover codes with the coverage area and percentage of the watershed.

| CORINE ID | SWAT Code | Land-use Definition | Area coverage (ha) | (%) |
|---|---|---|---|---|
| 112 | URMD | Discrete urban fabric | 178.8 | 0.23 |
| 121 | UIDU | Industrial units | 62.2 | 0.08 |
| 131 | UMES | Mining sites | 65.2 | 0.08 |
| 133 | URCS | Construction sites | 266.5 | 0.34 |
| 211 | AGRL | Rain-fed arable land | 26130.5 | 33.1 |
| 212 | CRIR | Permanent irrigated land | 477.7 | 0.60 |
| 222 | ORCD | Fruit trees and berry gardens | 1763 | 2.23 |
| 231 | PAST | Pastures | 69.5 | 0.09 |
| 242 | CRGR | Mixed agriculture patterns | 961.1 | 1.22 |
| 243 | CRDY | Agricultural land | 16686 | 21.1 |
| 311 | FODB | Deciduous forest | 27975.6 | 35.4 |
| 312 | FRSD | Evergreen forest | 118.7 | 0.15 |
| 313 | FRST | Mixed forest | 1128.8 | 1.43 |
| 321 | GRAS | Grasslands | 277.2 | 0.35 |
| 324 | TUWO | Conditional woodland-shrub | 2604.8 | 3.30 |
| 512 | WATR | Water areas | 200.2 | 0.25 |

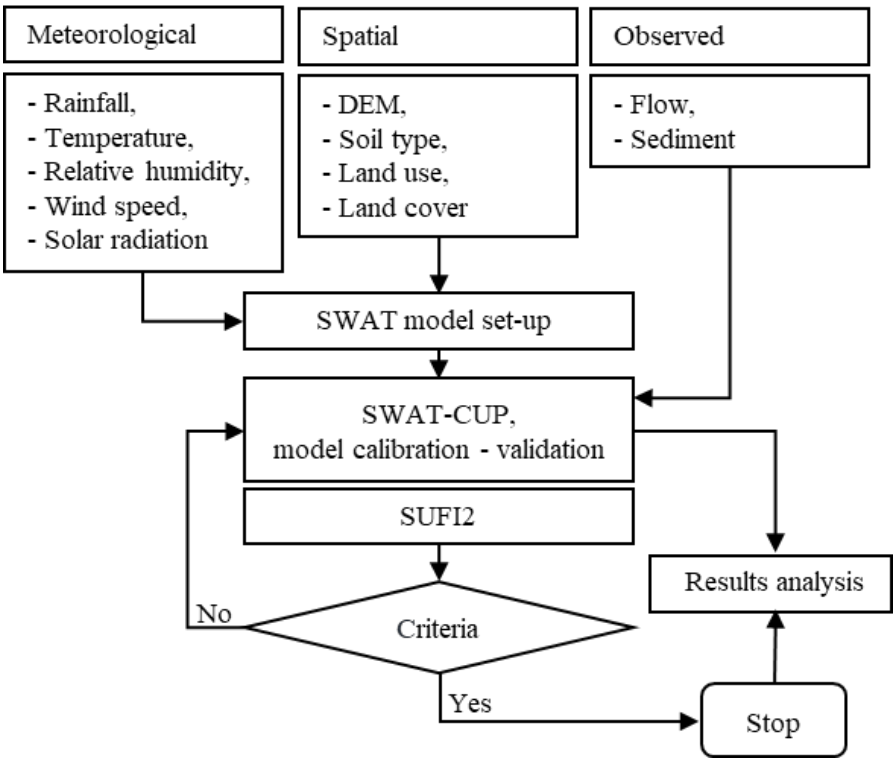


Figure 2. Flowchart of the fitting process

## Results and discussion

*Hydrological and sediment yield assessment*

In this study, for water balance modeling and the ability to perform a combined calibration-uncertainty analysis, the Sequence Uncertainty Fitting - version 2 (SUFI-2) algorithm (Abbaspour et al., 2007) is used for the model calibration. The parameter uncertainty in the SUFI-2 algorithm is calculated based on the uncertainty of the entire input and output sources, such as the uncertainty of input precipitation data, land-use, soil types, and measured data. The simulation uncertainty is quantified by the 95 Percent Prediction Uncertainty (95 PPU) and the P-factor. 95PPU is calculated at the 2.5% (Lower 95PPU) and 97.5% (Upper 95PPU) levels of the cumulative distribution function of the output variable obtained from the Latin hypercube sampling method, disallowing 5% of the very bad simulations. The relative width of the 95% probability band is the R-factor. Auto calibration and validation and uncertainty analysis of the model was performed monthly using the SUFI-2 method within SWAT-CUP software (SWAT Calibration and Uncertainty Procedures) (Abbaspour et al., 2000). Thirteen relative sensitivity value parameters were evaluated and determined during the parameter estimation process in this study. The hydrological and sediment models were calibrated and validated using observational data from two rainfall stations for 2005 to 2013 and 2014 to 2017. Two years of data are used as the warm-up period for the model calibration. Sensitivity analysis results indicated that the most sensitive hydrological and sediment parameters were the SCS runoff curve number (CN2) and channel erodibility factor (CH_COV1), respectively. For the hydrological model, the second group of parameters with similar significance was the base flow alpha-factor (ALPHA_BF), the groundwater delay (GW_DELAY), and the threshold water depth in the shallow aquifer (GWQMN). For the sediment model, the exponent parameter for calculating the channel sediment routing (SPEXP), the average slope length (SLSUBBSN), the linear parameter for calculating the channel sediment routing (SPCON), the soil erodibility factor in USLE (USLE_K), and the USLE support practice factor (USLE_P) were the following sensitive parameters.

This study uses $R^2$ and NSE as objective functions to assess the model predictions and performance. The NSE values for discharge and sediment were obtained as 0.85 and 0.63 for calibration and 0.94 and 0.42 for validation periods, respectively. Model predictions of discharge and sediment loads were also evaluated in terms of coefficient of determination ($R^2$ = 0.86 and $R^2$ = 0.65) for calibration periods, respectively. Although the flow model performed well, the consistency between the observed and simulated sediment loads during the validation period was not satisfactory (NSE <0.5). However, for the validation period, higher performance values of the flow model ($R^2$ = 0.94) and good sediment model performance ($R^2$ = 0.58) were obtained, which indicates that the estimation of the research process is favored.

The linear regression analysis was performed to assess the relationship between monthly measured flow and suspended sediment for the 2005-to-2017-time steps (Figure 3). The result shows the high correlation between the measured monthly flow and the suspended sediment with a coefficient of determination of 0.85.

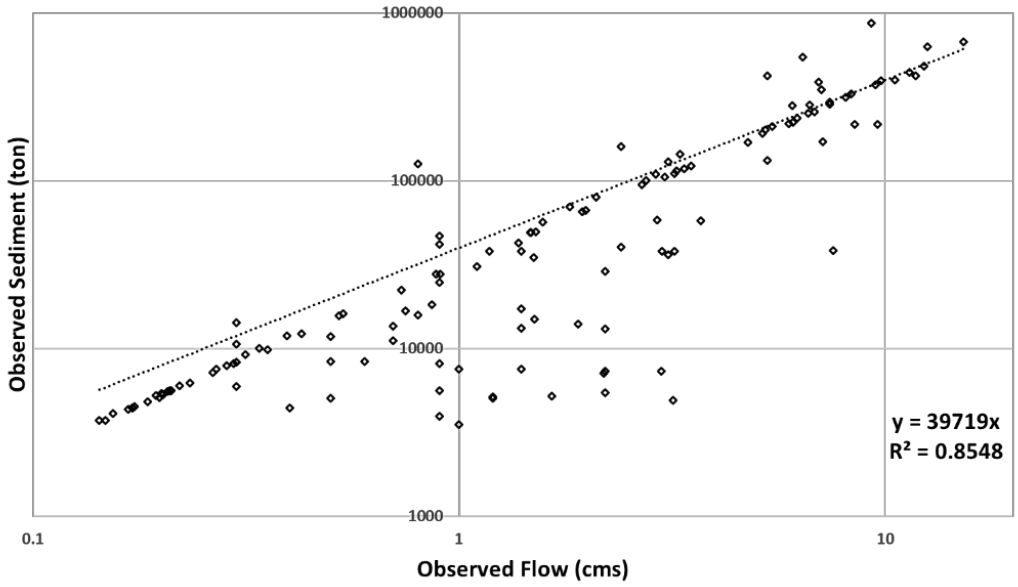


Figure 3. The relationship between monthly measured flow and suspended sediment (2005-2017)

## Deforestation impacts on discharge and sediment yield

According to the scenarios under consideration, the resulting forest burnt areas and converting these areas to farmland is approaching between 3500 and 11000 hectares, corresponding to the model execution from the minor deforestation (scenario 1) to the massive deforestation (scenario 3), respectively. According to the designed scenarios, the average percentage of deforestation following forest fires is 8%, and the maximum amount is up to 14% of watershed's total area. The effects of deforestation due to the forest fires and changing land-use type for agricultural activities and its impact on discharge and sediment was evaluated by modifying the model land-use type of the input data. To this end, forest patches (i.e., the FODB land-use type in the SWAT code, as shown in Table 2) and lands overlapped by considered area for deforestation and its spatial distribution have been changed for the non-irrigated arable land (i.e., the AGRL land-use type in the SWAT code) for each scenario (scenarios 1 to 3). These changes are introduced into the SWAT database to generate new land-use categories. Finally, the model was re-run for each scenario using the new inputs and parameters to assess discharge and sediments yield. The model results of all scenarios for the water balance components are summarized according to the annual time step, as shown in Table 3.

In each SWAT simulation, different output files are generated, such as the HRU output file (output.hru), the sub-basin output file (output.sub), and the main channel or reach section output file (output.rch). Table 4 provides a brief description of the output variables demonstrated in the output summary files in Table 3. The output summary files from the HRU's, subbasins, and streams have provided averaged amounts over the entire simulation period for deforestation scenarios of selected sub-watershed in the study area. The model output shows that in the range of variations in the values of different scenarios, in comparing with the baseline scenario, the average annual total water yield (i.e., the total amount of surface runoff, lateral flow, and baseflow) of the deforestation sub-watershed increased by 3 to 10%. Succeeding the increase in water yield, and due to the high erosiveness ability of excessive surface runoff, and land preparation operations for agriculture such as plowing, disc harrowing, rake and slope leveling or wedding, thus the sediment yield after the forest fires and changing into the farmland has a significant increase of 3.5 times in compared with the sediment yield before the land-use change. After a significant increase in sediment yield, organic nitrogen and phosphorus were two components that increased remarkably, especially in Scenario 3, where they were 2-fold and 2.5-fold higher, respectively, than the baseline scenario (no change in land-use).

Increases in organic nitrogen and phosphorus may result from the imbalance on their inputs and outputs' cycle. In addition, land use conversion (deforestation) to cropland leads to accumulation and increase of nitrogen and phosphorus sources from litter decomposition and soil mineralization. (Aber, 1992; Watson et al., 2000). In contrast, the actual evapotranspiration and leaf area index decreased by 5% and 24%, respectively, in selected sub-basins due to deforestation and the removal of trees and vegetation from the ground. Correspondingly, the total transported nitrogen, phosphorus, and sediment into the reach indicate a significant increase in the post-deforestation scenario. The amount of dissolved oxygen transported out of reach during the time step showed the inverse ratio and decreased 11 to 17% after deforestation compared to the baseline scenario.

In addition to the quantitative statistics (Table 3), Figures 4 and 5 also provide an intuitive comparison of the graphical representations and the spatial distribution of the total annual amounts of surface runoff and amount of water (water yield) and sediment (sediment yield) that leaves the sub-basins and contributes to streamflow in the reach before (baseline) and post-deforestation (scenarios 1 to 3) scenarios within the time step for the selected subbasins in the study area. The average annual

streamflow from each sub-basin and the amounts of sediment transported with water into the reach during the time step is also superimposed on the spatial distribution map of water and sediment yield, as shown in Figure 4 and Figure 5, respectively. Each sub-basin has a unique number assigned to it during the watershed delineation phase of the model.

Table 3. Based on the model output of deforestation scenarios, a summary of the water balance components in yearly time steps. Model outputs were obtained from scenarios and average hydrologic response to deforestation during the simulation period of 13 years (2005 to 2017).

| Outputs | Variables | Baseline | Scenario 1 | Scenario 2 | Scenario 3 | Variations (%) |
|---|---|---|---|---|---|---|
| HRU | SYLD | 758 | 863 | 896 | 1135 | 14-50 |
| | SEDP | 44 | 46 | 47 | 54 | 4-23 |
| | USLE | 1163 | 1247 | 1299 | 1557 | 7-34 |
| | ORGN | 237 | 260 | 264 | 311 | 10-31 |
| | ORGP | 40 | 46 | 47 | 56 | 13-39 |
| | LAI[b] | 123 | 120 | 119 | 93 | 24-2 |
| SUB | WYLD | 3897 | 4003 | 4074 | 4265 | 3-10 |
| | SURQ | 1877 | 1997 | 2082 | 2325 | 6-24 |
| | SYLD | 381 | 741 | 949 | 1702 | 95-347 |
| | SEDP | 28 | 39 | 46 | 70 | 40-153 |
| | ORGN | 159 | 239 | 292 | 458 | 50-188 |
| | ORGP | 26 | 44 | 56 | 91 | 70-254 |
| | ET[b] | 6502 | 6421 | 6351 | 6167 | 5-1 |
| RCH[a] | SED_IN | 0.51 | 1.13 | 1.38 | 2.25 | 121-341 |
| | TOT_N | 2.6 | 2.66 | 2.79 | 3.28 | 2-26 |
| | TOT_P | 0.87 | 0.89 | 0.94 | 1.1 | 2-27 |
| | DISOX[b] | 1.02 | 0.9 | 0.87 | 0.85 | 17-11 |

[a] All values in baseline and scenarios are multiplied by $10^6$, [b] There is an inverse ratio.
*The values in the table are presented for the selected sub-watershed of the study area.

Table 4. A brief description of the output variables that have been demonstrated in the output summary files (Arnold, 2012)

| Outputs | Variable name | Definition |
|---|---|---|
| HRU | SYLD | Sediment yield (metric tons/ha) |
| | SEDP | Mineral phosphorus absorbed to sediment (kg P/ha) |
| | USLE | Soil loss calculated with the USLE* equation (metric tons/ha) |
| | ORGN | Transported amount of the organic nitrogen out of the HRU and into the reach (kg N/ha) |
| | ORGP | Transported amount of the organic phosphorus with sediment into the reach (kg P/ha) |
| | LAI | Leaf area index at the end of the time step |
| SUB | WYLD | Water yield, the total net amount of water that exit the sub-basin (mm H2O) |
| | SURQ | Surface runoff contribution to streamflow during the period (mm H2O) |
| | SYLD | Sediment yield (metric tons/ha) from the subbasin |
| | SEDP | Mineral phosphorus attached to sediment (kg P/ha) |
| | ORGN | Transported amount of the organic nitrogen out of the sub-basin and into the reach (kg N/ha) |
| | ORGP | Organic phosphorus transported with sediment into the reach (kg P/ha) |
| | ET | Sub-basin actual evapotranspiration during the time step (mm) |
| RCH | SED_IN | Transported sediment with water into reach during the period (metric tons) |
| | TOT_N | Total surface runoff nitrogen (kg) |
| | TOT_P | Total surface runoff phosphorus (kg) |
| | DISOX_IN | Transported dissolved oxygen into reach during the period (kg O2) |

*Universal Soil Loss Equation

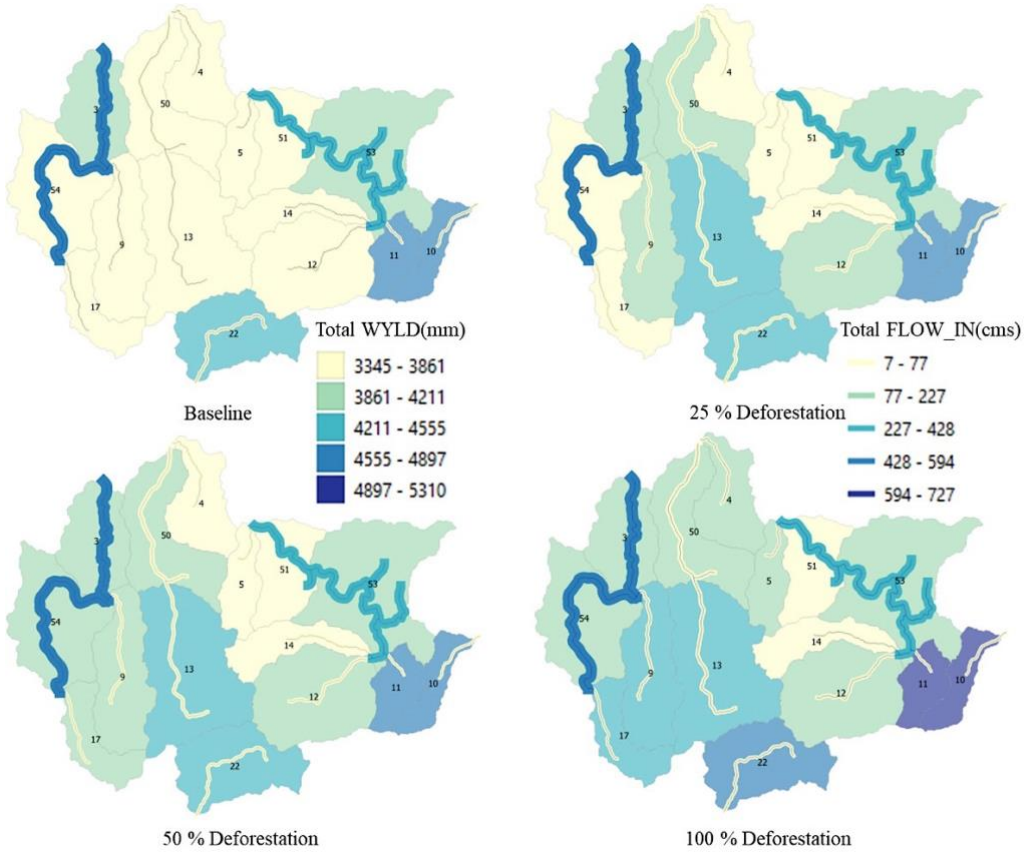


Figure 4. The total annual amounts of water yield for baseline and post-deforestation (scenarios 1 to 3) scenarios at the sub-catchment scale in time step (2005-2017)

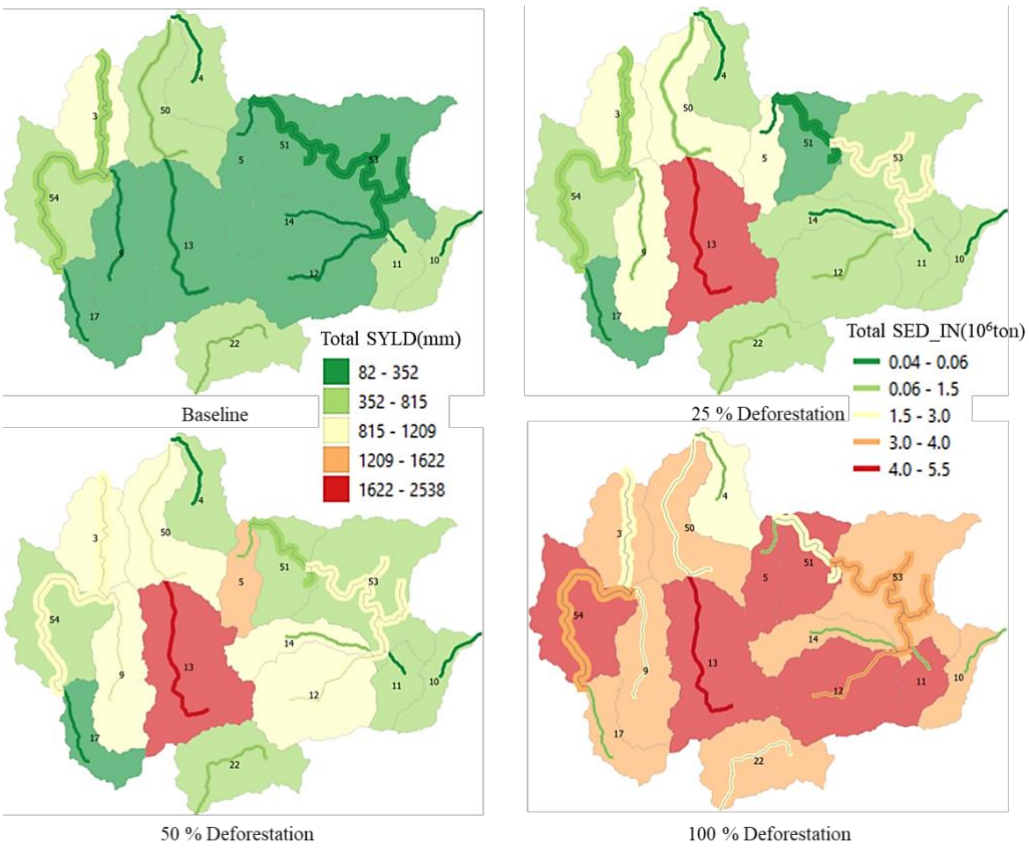


Figure 5. The total annual amounts of sediment yield for baseline and post-deforestation (from 1 to 3) scenarios at the sub-catchment scale in time step (2005-2017)

The progressive increase of deforestation area brings along a gradual increase in the annual mean flow rates, and the maximum runoff occurred in scenario 3 with the highest spatial distribution of deforestation where the surface runoff increased by 24% in compared with the baseline scenario and has raised from about 144 mm to 179 mm. In consequence of the degradation of forest and conversion, it into the farmland, a high contribution of the surface runoff to the streamflow and a significant increase in the amount of sediment transported into the stream has been observed that indicates a large amount of soil loss has occurred during the simulation time step. The amount of sediment eroded from HRUs and transported to the hydrological network of the selected sub-basins varied between 64.3- and 87.3-ton ha-1. Post-deforestation conditions resulted in an almost 50% increase compared with the baseline scenario, corresponding to an erosion rate increase of 29-ton $ha^{-1}$. Accordingly, soil loss from HRUs calculated with the USLE equation between the baseline and the scenario with the highest distribution of deforestation (scenario 3) changed from 89.5 to 119.8 (ton) and increased by 34% for the time step.

Erosion rates and sediment yield analysis identify areas with very high soil losses and their spatial distribution. The highest sedimentation rates were estimated particularly at sub-basins 5, 11-13, 51, and 54 of the selected area. Different parameters such as soil type and land topography (land on steep slopes) in the area can affect the erosion process and soil loss and cause to increase in soil erosion rate and sediment loss under high runoff conditions. According to the post-deforestation scenario analysis, the sedimentation rate of almost all distribution areas is very high, and the erosion rates would be above the tolerable soil losses limit (Verheijen et al., 2009). This result indicates that the selected sub-watershed has different areas where soil erosion is a substantial issue, and land degradation would be possibly higher than the soil formation rates in the watershed.

Model predictions and outputs indicate that after changes in land use/land cover due to deforestation, there is a high possibility of soil loss, sedimentation, and an increase in surface runoff. The hydrological and sediment model's outputs suggest that there are problems in landscape susceptibility to erosion and soil loss, and it is necessary to identify areas in the watershed that are at greater risk of erosion when land use/cover changes occur. Hence, the method used in this study can be helpful for decision-makers and an efficient approach to apply for the protection of forests from intensive impacts such as forest fires or deforestation.

## Conclusion

Assessing the impact of land use/cover changes and deforestation on the hydrological response of watersheds is a critical step in reducing the likelihood of excessive surface runoff and erosion rates after deforestation. The development of hydrological models can effectively help management practices deal with the adverse effects of deforestation, such as soil erosion and sedimentation. This study investigated the applicability of the SWAT rainfall-runoff model to assess the hydrological consequences of land use/cover changes (i.e., deforestation) and their effects on surface runoff, erosion, and sediment load under baseline and post-deforestation scenarios. The results showed that the hydrological budget of the selected sub-basins has changed significantly in the post-deforestation period. In the deforestation scenario with the highest spatial distribution, due to the high erosivity (24% increase) of excessive surface runoff after deforestation, sediment yield increased 3.5 times. This study recommends the potential use of hydrological and erosion models to analyze the impact of deforestation on sediment load under different scenarios. The consequences of deforestation on hydrological services can help decision-makers choose better post-deforestation mitigation plans to limit the impact of the forest degradation on the sediment load as well as its environmental issues.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared no actual potential or perceived conflict of interest for this research article.

### Author contribution

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

### Ethical approval

Ethics committee approval is not required.

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### Data availability

Not applicable.

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Not applicable.

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# Genetically modified organisms and biosafety as perceived by professionals according to their sociodemographic characteristics

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## Abstract

While the cultivation of modern biotechnology products was adopted by producers very fast, the consumer acceptance of these products has not evolved at the same pace. Despite multiple benefits associated with genetically modified organisms (GMO), some potential risks related to the environment, health and economy have increased concerns and affected consumer confidence. This article examines the relationship between knowledge and confidence levels for biosafety and GMOs, and the sociodemographic characteristics of professionals working in the fields of food, agriculture and veterinary medicine, with a view to assess their perception. Questionnaires were applied to 261 individuals, selected by the proportional sampling method among professionals, the numbers of whom were obtained from the relevant unions and chambers. Then frequency, percentage distribution and factor analyses were carried out on data obtained. In conclusion food engineering professionals, men and more knowledgeable participants in biotechnology were found to have a more positive approach towards GMOs and more confidence in the biosafety system. It is indisputable that the scientific knowledge is essential for a reliable biosafety mechanism. Therefore, the inclusion of courses on GMOs and biosafety into curricula and planning in-service trainings may significantly contribute to the development of scientific knowledge thus, improving attitudes towards GMOs.

## Keywords

Biosafety, Food biotechnology, GMO trust and confidence, GMO knowledge, Consumer perception

## Introduction

As of the late 20th century, given its highly advanced level and scope of application, modern biotechnology has become an added value of leading edge technologies. While having brought about favourable economic and social transformations in many sectors, it has also raised question marks (Yilmaz, 2014). Although biotechnology products are offered as a solution to the global hunger problem and are claimed to be more efficient, more economical and environmentally friendly, discussions continue on their adverse impact on the environment and biodiversity, as well as the potential health risks they pose, including allergies, antibiotic resistance and toxicity (Cebi & Olhan, 2019).

Existing regulations on GMOs across the world are handled as either non-binding, guide-based international regulations or binding legal regulations. Among them, the United Nations Convention on Biodiversity and the Cartagena Biosafety Protocol are the main internationally binding texts to which Türkiye is also a party (Soykan, 2007). Whereas Türkiye is not a party yet in the Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to the Convention on Biological Diversity and entered in force in 2018. (Karakoc Yildiz, 2019).

There are also contextual differences between texts regulating the trade of GMOs such as the Cartagena Biosafety Protocol and the World Trade Organization (WTO) agreements. The main purpose of the WTO is to support free trade (Aksu, 2019), while in the Cartagena Biosafety Protocol, which is an annex to the Biodiversity Convention, the main objective is to

prevent the adverse effects of GMOs on biodiversity, such that the precautionary principle has been adopted (Ates, 2020).

Two different perceptions of GMOs have created different legal frameworks in countries. While the USA implements its policies and regulations based on the principle of "basic equivalence", considering GMO and traditional products to be similar, the EU is more cautious and considers GMO products to be different from traditional ones, based on the precautionary principle (Karakoc Yildiz, 2019). Differences reflected in the legal regulations are also came out in the production and trade of these products. While countries such as the USA, Brazil, Canada and Argentina are the leaders in the production of GMO products, EU countries do not favour the production and trade of these products, with a few exceptions. The top ten countries producing GMOs in the world produced transgenic plants on an area of more than 1 million hectares in 2019. As can be seen in Table 1, among these top ten countries, only the USA and Canada are classified as developed countries, while the rest are classified as developing (ISAAA, 2019).

Table 1. Global transgenic plant cultivation in 2019

| Rank | Country | Area (million hectare) | Commercial Transgenic Products |
|---|---|---|---|
| 1 | USA | 71.5 | Maize, soybean, cotton, alfalfa, canola, sugar beet, potato, papaya, squash, apple |
| 2 | Brazil | 52.8 | Soybean, maize, cotton, alfalfa |
| 3 | Argentina | 24.0 | Soybean, maize, cotton, alfalfa |
| 4 | Canada | 12.5 | Canola, soybean, maize, sugar beet, alfalfa, potato |
| 5 | India | 11.9 | Cotton |
| 6 | Paraguay | 4.1 | Soybean, maize, cotton |
| 7 | China | 3.2 | Cotton |
| 8 | South Africa | 2.7 | Maize, soybean, cotton |
| 9 | Pakistan | 2.5 | Cotton |
| 10 | Bolivia | 1.4 | Soybean |

Source: (ISAAA, 2019)

In recent years, some new breeding techniques, such as genome editing, have allowed scientists to improve the characteristics of living organisms. The technologies most commonly used in genome editing are clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases or mega nucleases (ISAAA, 2022). CRISPR-Cas technology allows making changes in targeted regions of human, animal, plant and microorganism's genetic material (Tastan, 2018). The two types of legal approaches mentioned above also come to the fore in determining whether or not to extend the controls applied to GMOs to commercial varieties obtained with novel methods such as CRISPR. While the techniques used in the development of new varieties have been taken into account with a process-based approach in the EU, Argentina, Brazil and several other countries, a product-based approach has been adopted by the USA and Canada (Akbudak & Kontbay, 2017).

In addition to all these regulatory studies on GMOs, several scientific studies have aimed to assess the consumers' perspectives on GMOs. In a study dating back to 2003, it was stated that consumers' perspectives and attitudes towards GMOs changed positively in direct proportion to their education and knowledge level (Hossain et al., 2003). Aleksejeva (2014) again, showed that EU Experts, who have a high level of knowledge about GMOs and are involved in the decision-making process, support the use of GMOs in food and feed. There are also studies showing that consumers' attitudes towards GMO foods vary among countries and individuals (Rodríguez-Entrena & Salazar-Ordóñez, 2013). For example, the attitude of US citizens towards GMOs is more positive than that of Canadians, European Union citizens and Irish. (Gaskell, 2005; Wolf at all., 2004; Tukelman, 2017). In a study conducted in the rural areas of China in 2015, it was determined that consumer attitudes towards GMOs were shaped by subjective comments rather than objective information, and beneficial perspectives on these products positively affected purchasing behaviour (Liu & Zhang, 2015). On the otherhand, the research conducted with approximately thirty thousand participants in the EU in April 2019 showed that the Europeans' concerns about GMOs had decreased, such that the percentage of respondents identifying 'genetically modified ingredients in food or drinks' as a concern, which was 66% in a 2010 survey, had fallen to 27% in the 2019 (EFSA, 2019).

Legislation on GMOs in Türkiye consists of the Convention on Biological Diversity, the Cartagena Biosafety Protocol and the Biosafety Law with three regulations at the national level (Civgin, 2013). According to the Biosafety Law numbered 5977 (2010), which prohibits the entry of genetically modified seeds into Türkiye, it is forbidden to place GMOs and their products on the market without approval, to use or make use of GMOs and their products in violation of prevailing decisions, to cultivate genetically modified plants and produce genetically modified animals, to use GMOs and their products outside their defined scope and purpose, and to use GMOs and products in baby foods and infant formulas, follow-up formulas, follow-on formulas and supplementary foods for infants and young children. In addition, with amendments made to the Biosafety Law over time, the Biosafety Board was abolished and the Ministry of Agriculture and Forestry assumed its duties (TBBDM, 2022). In a study

conducted by Civgin (2013), it is stated that the current GMO legislation in Türkiye is overall compatible with the Convention on Biological Diversity and the Cartagena Biosafety Protocol.

In line with all these developments, the perspectives of both consumers and experts on biotechnological advancements have been investigated through many studies in Türkiye. The research conducted in three different regions of Türkiye (Southeast/Eastern Anatolia, Aegean and Black Sea) in 2012 found that while 73% of the participants stated that they had heard of the concept of GMO, 27% stated that they had never heard the term before (Baykan & Ertunc, 2012). On the other hand, Temelli and Kurt (2011) concluded that students did not have adequate scientific knowledge on GMOs, and were therefore cautious about the use of GMOs, which highlighted the need to increase their information level. Adana et al. (2014) found that nursing and midwifery students did not have sufficient knowledge about genetically modified organisms. Merdan (2019) conducted a study revealing that the socioeconomic characteristics of students significantly defined their level of knowledge about GMO products.

The hypothesis "the level of knowledge and confidence of professionals working in the fields of food, agriculture and veterinary medicine about biosafety and GMOs is high" was aimed to be set out in this study. The attitudes of food engineering, agricultural engineering and veterinary medicine professionals, who are thought to have a high level of knowledge about biosafety and GMOs, were determined and differences in the knowledge and confidence levels of these professionals in relation to their sociodemographic characteristics were demonstrated. This research contributes to determining the need for the development of training programs addressing public concerns over GMOs by the relevant authorities for a reliable and effective biosafety mechanism.

## Materials and Methods

In order to identify the main population of the research, the numbers of food engineers, veterinarians and agricultural engineers working in the Ankara Province were obtained from the Chamber of Food Engineers, the Chamber of Agricultural Engineers and the Veterinary Medical Association on August 4, 2015. The proportional sampling approach was used to determine the number of subjects to be sampled (Aksoy & Yavuz, 2012). Accordingly, 261 individuals were selected among 8570 professionals working in Ankara with the proportional sampling method with a confidence level of 90% and a 5% margin of error.

Questionnaires were applied to professionals working for the Ministry of Agriculture and Forestry in Ankara Province and responses were obtained through software after preliminary interviews with the individuals. Collection of all questionnaires were completed in 2021. Accordingly, the official figures obtained are shown in Table 2.

Table 2. Sample Distribution

| Group of Profession | Number of Professionals | Rate in Total | Number of Professionals to be Surveyed |
|---|---|---|---|
| Food Engineering | 1224 | 14% | 37 |
| Veterinary Medicine | 2031 | 25% | 64 |
| Agricultural Engineer | 5315 | 61% | 160 |
| Total | 8570 | 100% | 261 |

The first part of the questionnaire applied in the present study comprised of questions to measure the sociodemographic characteristics of the participants, and the second part included the statements to determine their general thoughts on biosafety and GMOs. The SPSS26 software was used in the analyses of the obtained data. A 3-point Likert attitude scale (1 = disagree, 2= indecisive, 3= agree) was used, frequency and percentage analyses of the questionnaire responses were performed, followed by an explanatory factor analysis (Kline., 1994; Buyukozturk, 2002).

The Kayser-Meyer-Olkin (KMO) value, comparing the magnitudes of the observed correlation coefficients in relation to the magnitudes of the partial correlation coefficients was used to measure the sampling adequacy and the Bartlett spherical value was used to test the hypothesis that the correlation matrix is an identity matrix. In determining the number of factors in this research, the sum of the squares of the factor loads of each factor, the eigenvalues (coefficient used in calculating the ratio of the variance explained by each factor and deciding the number of important factors) were taken into account. In the factor analysis, in which the variance explained by the factor increases as the eigenvalue increases, the factors with an eigenvalue of 1 were taken as important factors. The naming of the factors was based on the common characteristics of several factors. The Cronbach alpha coefficient was used for the internal consistency of the data set and the Kolmogorov Smirnov test was used to determine the distribution of the variables and showed that they did not exhibit a normal distribution (Tabachnick, B. G., & Fidell, L. S., 2007; Buyukozturk, 2002). The Mann-Whitney U test was used for the comparison of two groups, and the Kruskall-Wallis H-test was used for the comparison of three or more groups, as non-parametric analysis techniques (Akdag, 2011).

## Results

### Descriptive Statistics on the Sociodemographic Characteristics

The first part of the questionnaire aimed to determine the sociodemographic characteristics including socioeconomic statistics of the participants. According to the frequency and percentage distributions of the sample from which the data were obtained, the ages of the participants, whose male and female ratios were similar, varied between 31-51 years and above. Nearly half of the participants held master of science degrees in either agricultural engineering, veterinary medicine or food engineering. It was determined that the attendance

of the participants to biotechnology courses was at a level higher than that to GMO-specific courses. Furthermore, about half of the participants had also attended biotechnology trainings due to their job. The percentage distribution of the data obtained in the research is shown in Table 3.

Table 3. Statistics of sociodemographic characteristics

| **Criteria** | **Category** | **Total** | **%** |
|---|---|---|---|
| Age | 26-30 | 10 | 3.8 |
| | 31-40 | 120 | 46 |
| | 40-50 | 48 | 16.5 |
| | 51 + | 88 | 33.7 |
| | **Category** | **Total** | **%** |
| Gender | Male | 135 | 51.7 |
| | Female | 126 | 48.3 |
| | **Category** | **Total** | **%** |
| Occupation | Agricultural Engineer | 160 | 61.3 |
| | Veterinarian | 64 | 24.5 |
| | Food Engineer | 37 | 14.2 |
| | **Category** | **Total** | **%** |
| Household Size | 1-3 | 149 | 57.1 |
| | 4-6 | 111 | 42.5 |
| | 6 + | 1 | 0.4 |
| | **Category** | **Total** | **%** |
| Who do you live with right now? | Alone | 17 | 6.5 |
| | With spouse | 43 | 16.5 |
| | Elementary family | 110 | 42.1 |
| | Extended family | 74 | 28.4 |
| | Others | 17 | 6.5 |
| | **Category** | **Total** | **%** |
| If you have children, their age? | No child | 25 | 9.5 |
| | 0-24 mounth-old child | 47 | 18 |
| | 2-13 year-old child | 141 | 54 |
| | 13-18 year-old child | 38 | 14.5 |
| | 18 + | **10** | **4** |
| | **Category** | **Total** | **%** |
| Educational background | Undergraduate | 97 | 37.2 |
| | Graduate | 120 | 46 |
| | Doctorate | 44 | 16.9 |
| | **Category** | **Total** | **%** |
| Monthly salary (%) | 4000-5000 TL | 11 | 4.2 |
| | 5001-7000 TL | 57 | 21.8 |
| | 7000 TL + | 193 | 73.9 |
| | **Category** | **Total** | **%** |
| Budget for food | 500 TL and below | 6 | 2.3 |
| | 500-1000 TL | 37 | 14.2 |
| | 1000-2000 TL | 112 | 42.9 |
| | 2000 TL + | 106 | 40.6 |
| | **Category** | **Total** | **%** |
| Have you taken a course on biotechnology? | Yes | 156 | 59.8 |
| | No | 105 | 40.2 |
| | **Category** | **Total** | **%** |
| Have you taken a course on GMOs and GMO products? | Yes | 99 | 37.9 |
| | No | 162 | 62.1 |
| | **Category** | **Total** | **%** |
| Have you attended trainings on biotechnology? | Yes | 120 | 46 |
| | No | 141 | 54 |

### Statistics on the Participants' Perspectives on Biosafety and GMOs

Frequency and percentage analyses of the expressions of the participants on GMOs and the current biosafety policy were performed using a 3-point Likert attitude scale. Accordingly, it was determined that half of the participants were misinformed on the presence of GMOs on the national market, but the majority of them knew that GMOs were available for feed purposes on the national market. Again, nearly half of the participants didn't support the consumption of products such as meat, milk and eggs from animals fed on GMO feed. The development of GMO products (e.g., foods with increased nutritional value), despite proven positive effects on the consumer was also not supported by half of the participants. On the other hand, it was shown that most of the participants were disinclined to consume GMOs or GMO products as food, even if they were cheaper than the non-GMO equivalent of the same product, and were opposed to the production of genetically modified animals (such as cattle with increased milk yield). More than half of the participants were in favour of the production of health products such as vaccines. Again, according to the aforementioned data, more than half of these participants, who had the habit of checking product labels, had not come across with food products labelled as a GMO or GMO product, but stated that they may have consumed a GMO or its product for food purposes unwittingly. When the question about biosafety and the level of knowledge about GMOs and their products was asked, the participants gave three different answers, and no clear distinction was observed between agreeing, disagreeing and being indecisive. The opinions of the participants regarding all statements are shown in Table 4.

Table 4. Opinions of the Participants on Biosafety and GMOs

| Expressions | Disagree | | Indecisive | | Agree | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Frequency (*f*) | Rate (%) | Frequency (*f*) | Rate (%) | Frequency (*f*) | Rate (%) | Frequency (*f*) | Rate (%) |
| There are GMOs and/or their products for use as food on the national market. | 96 | 36.8 | 35 | 13.4 | 130 | 49.8 | 261 | 100 |
| There are GMOs or products for use as feed on the national market. | 8 | 3.1 | 14 | 5.4 | 239 | 91.6 | 261 | 100 |
| There is no harm in consuming products such as meat, milk, eggs obtained from animals fed with GMO feed. | 116 | 44.4 | 95 | 36.4 | 50 | 17.2 | 261 | 100 |
| I support the development of GMO products (e.g., nutritionally enhanced foods) with proven positive effects on the consumer. | 135 | 50.8 | 72 | 27.6 | 54 | 20.7 | 261 | 100 |
| If a GMO or GMO product is cheaper than its non-GMO equivalent, I would consume it as food. | 197 | 75.4 | 42 | 16.1 | 22 | 8.4 | 261 | 100 |
| In Türkiye, genetically modified animals (such as cattle with increased milk yield) should be produced, if permitted by law. | 157 | 60.2 | 56 | 21.5 | 48 | 18.4 | 261 | 100 |
| Health products such as vaccines should be produced using gene technology in Türkiye. | 35 | 13.4 | 52 | 19.9 | 174 | 66.6 | 261 | 100 |
| I check the labels of the products I buy. | 12 | 4.5 | 3 | 1.1 | 246 | 94.2 | 261 | 100 |
| While shopping at the market, I came across with products sold for food purposes with a label stating that the product was a GMO or GMO product. | 160 | 61.3 | 63 | 24.1 | 38 | 14.5 | 261 | 100 |
| In Türkiye, I may have consumed a GMO or its product for food purposes unwittingly. | 40 | 15.3 | 51 | 19.5 | 170 | 65.2 | 261 | 100 |
| I think that the chicken whose meat I consumed was fed with GMO feed. | 18 | 6.8 | 51 | 19.5 | 192 | 73.5 | 261 | 100 |
| I have sufficient knowledge of biosafety and GMOs and their products. | 84 | 32.2 | 90 | 34.5 | 87 | 33.3 | 261 | 100 |

### Participants' GMO Perception According to their Sociodemographic Characteristics

In line with the information obtained, four different types of scores were created for the following statements: "positive view on GMOs", "need to be informed on GMOs", "trust in the biosafety system" and "legislative knowledge". A factor analysis, based on dimension reduction, was used for the creation of these scores. The results obtained by factor analysis are shown in Table 5. For the data set to be considered suitable for factor analysis, the KMO value should be above 0.50 and the Bartlett Sphericity Test result should be statistically significant ($p<0.05$) (Buyukozturk, 2002).

The calculation of the KMO value as 0.755 showed that the sample size was sufficient for factor analysis. On the other hand, as a result of the Sphericity Test, the

p-value=0.00 <0.05. Accordingly, the data were suitable for factor analysis. As seen in Table 5, the total variance explained by these four factors regarding the scale was 56.3%. Since Cronbach's alpha values indicated high, rather high or very high reliability levels for all the questions collected under the factors, overall the scale was found to be highly reliable. The factor loads, Cronbach's alpha reliability coefficients, the total explained variance, which is an indicator of the related concept or structure having been measured well, and the data of the KMO and Bartlett Sphericity Tests are shown in Table 5.

Table 5. Factor Loads and Alpha Confidence Coefficients

| Dimensions | Cronbach Alpha | Factor Loads |
|---|---|---|
| **positive view on GMOs** | **0.752** | |
| I support the development of GMO products (e.g., nutritionally enhanced foods) with proven positive effects on the consumer. | | 0.835 |
| If a GMO or GMO product is cheaper than its non-GMO equivalent, I would consume it as food. | | 0.782 |
| In Türkiye, genetically modified animals (such as cattle with increased milk yield) should be produced, if allowed by law. | | 0.757 |
| There is no harm in consuming products such as meat, milk, eggs obtained from animals fed with GMO feed. | | 0.717 |
| **need to be informed on GMOs** | **0.927** | |
| There are GMOs or GMO products for use as food on the national market. | | 0.794 |
| In Türkiye, I may have consumed a GMO or its product for food purposes unwittingly. | | 0.618 |
| While shopping at the market, I came across with products sold for food purposes with a label stating that the product was a GMO or GMO product. | | 0.611 |
| **trust in the biosafety system** | **0.854** | |
| The Biosafety Board can take impartial decisions. | | 0.732 |
| Criminal sanctions in our Biosafety Law are deterrent. | | 0.693 |
| The Biosafety Law ensures the protection of our biodiversity. | | 0.692 |
| **legislative knowledge** | **0.821** | |
| According to our biosafety legislation, there is a labelling requirement for GMOs and their products. | | 0.671 |
| According to our Biosafety Law, experimental GMO production is allowed. | | 0.582 |
| According to our Biosafety Law, the production of genetically modified plants and animals is prohibited. | | 0.568 |
| **All questions** | **0.836** | |
| **Eigenvalue** | | **1** |
| **Factor Variance (%)** | | **56.348** |
| **KMO Value** | | **0.755** |
| **Bartlett Sphericity Test** | | **p =0.00 < 0.05** |
| **Cronbach Alpha=** | | **0.836** |

Subsequently the Kolmogorov-Smirnov test was applied to the four different scores to determine whether the sample in question came from a normal distribution. According to the results obtained for the need for information on GMOs, a positive view of GMOs, trust in the biosafety system and legislative knowledge, it was determined that the variables did not have a normal distribution. At this point, the non-parametric Mann-Whitney and Kruskal-Wallis tests were used (Buyukozturk, 2002). In the score types created, the differences were tested for gender, occupation, education level (taking biotechnology courses, taking GMO courses, attending biotechnology trainings for work), people with whom they live, income level, and the budget allocated for food. The differences for each of the mentioned score types are summarized in Table 6, such that situations, in which no difference in all variable types was observed, were not included in the table.

Table 6. Differences in the score types according to sociodemographic characteristics

| Variable | The need to be informed on GMOs | Positive view on GMOs | Trust in the Biosafety System | Legislative Knowledge |
|---|---|---|---|---|
| **Gender** | No difference | Men scored higher (p=0.018) | No difference | No difference |
| **Occupation** | Food engineers scored lower (p=0.00) | No difference | Food engineers scored higher (p=0.01) | Food engineers scored higher (p=0.00) |
| **Level of Education** | No difference | No difference | Those holding a doctorate degree scored higher (p=0.051) | No difference |
| **Status of Prior Attendance to Biotech. Course** | No difference | Those who had taken courses scored higher (p=0.061) | Those who had taken courses scored higher (p=0.059) | Those who had taken courses scored higher (p=0.004) |
| **Status of Prior Attendance to Courses on GMOs** | No difference | No difference | Those who had taken courses scored higher (0.033) | No difference |
| **Status of Prior Attendance to Biotech. Course for Work** | Those without prior training scored higher (p=0.06) | Those with prior training scored higher (p=0.008) | Those with prior training scored higher (p=0.076) | Those with prior training scored higher (p=0.000) |

## Discussion

This study investigated the knowledge and confidence levels of food engineering, agricultural engineering and veterinary medicine professionals for GMOs and the national biosafety policy, and the sociodemographic factors affecting their perceptions. It was determined that, overall, almost half of the participants showed a supportive approach to GMOs in their statements. However, more than half of the participants were sceptical of the statements based on trust. Previous research has shown that the people of different countries develop prejudices due to lack of information about GMOs and various psychosocial and economic reasons, and since no definite judgement can be made about the knowledge levels of these societies, it has been concluded that the determinants of the attitudes and behaviours of the people are the psychosocial priorities of that society and the individual (Atikcan et al., 2011).

In this study men showed a higher percentage of positive viewpoint towards GMO than women. In fact, similar to our findings, in previous studies women were found to be more concerned about GMO ethics and health aspects than men (Subrahmanyan and Cheng, 2000; Tukelman, 2017).This study also revealed that the need for information about GMOs is less and the positive point of view is higher of food engineers. It has also been determined that the scores of food engineers are high in the dimensions of trust in the biosafety system and knowing the legislation. Atikcan et al. (2011) examined the awareness of students in food engineering and other faculties in Türkiye in terms of demographic characteristics such as gender and determined that men were more aware of GMOs and food engineering students were more knowledgeable since they are educated on this subject. Furthermore, in this study, it was determined that those holding a doctorate degree had a higher level of trust in the biosafety system. It has already been determined that a high level of scientific understanding leads to fewer negative opinions, higher acceptance rates, and less discrimination among types of genetic modification (McComas et al., 2014). In this study, it was proved that the participants who had taken courses on biotechnology had a more positive attitude towards GMOs, were more confident in the biosafety system, and had more knowledge on legislation. Participants who had taken a course on GMOs trusted these products more. Statistically significant differences were observed for all score types for professionals had participated in biotechnology trainings for work. In fact, as mentioned above, it is known that participants with a high level of scientific knowledge have less negative thoughts about GMOs (Wunderlich & Gatto, 2015). In a study conducted to measure the knowledge and attitudes of nursing students about GMOs, it was determined that they considered GMOs to be very risky despite their low knowledge levels, and it was stated that adding GMO-related courses to the curriculum would contribute to improving their knowledge and attitude (Turker et al., 2013). In another study conducted on consumers in 2003, it was stated that their perspectives and attitudes towards biotechnological applications and products changed in direct proportion to their education and knowledge levels (Hossain et al., 2003). Yet in Aleksejeva's (2014) research, highly knowledgeable EU experts, who are involved in the decision-making process for GMOs, support the use of GMOs in food and feed. Thus, as Yilmaz V. (2020) stated a reliable and effective biosafety mechanism is essential for addressing the concerns of the society over GMO products.

## Conclusion

Among the population of the present study food engineering professionals, men and more knowledgeable participants in biotechnology were found to have a more positive perception of GMOs and be more confident in the biosafety system. Increasing trust in the biosafety system depends on eliminating information pollution and increasing the level of scientific knowledge. Thus, inclusion of courses on GMOs and biosafety into curricula and planning in-service trainings may significantly contribute to the reliability of the biosafety mechanism.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

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# Essential oil content and chemical profile of *Cotinus coggygria* Scop. from Eastern Anatolia Türkiye

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## Abstract
The essential oil of the herb of the medicinal plant known as "smoke tree" (*Cotinus coggygria* Scop.) from Eastern, Anatolia (Turkey) was analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). Twenty ingredients were identified for flowers representing 100% of the total oil while eleven ingredients were characterized in leaf essential oil comprising 99.96% of the oil extracted. As thymol (31.33%) and carvone (20.57%) were comprising main ingredients of the flower oil, the major ingredients of the leaf were determined as limonene and cis-ocimene by 48.53% and 23.57%, respectively. Identification of thymol form leaf sample as the predominant ingredient was first reported.

## Keywords
*Cotinus coggygria* Scop., GC/MS, Essential oil, Thymol, Carvone

## Introduction
*Cotinus coggygria* is one of the smallest genus of the *Anacardiaceae* family and is a species defined as a the "Smoke tree". In addition, the plant, which has a wide distribution area, stretches from the Mediterranean, Southern Europe, Moldova, the Himalayas and the Caucasus to Central China (Novakovic et al., 2007; Tunç et al., 2013; Banadkooki et al., 2019). It is frequent in some parts of Turkey mainly North, East and South biomes, called "Boyacı sumağı", "Sarı boya", "Sarı can" and "Sarı yaprak", locally (Bakış et al., 2011).

Generally, the plant is regarded as either wide shrubs or small tree, and has simple, oval leaves of yellow colour with a length of 3-8 cm, 18 cm thick. The flowers of the pentamerous plant, sometimes pale yellow or yellow-green, and abortive-hermaphrodite, long peduncle, in pole free blooming (da Silva et al., 2018; Pandourska et al., 2021; Shaboyan et al., 2021).

*C. coggygria* is a beneficial plant known for its medicinal activity, useful timber and landscape appeal. The young branches and leaves of the plant are used for the production of the aforementioned terpenic fragrant essential oil for use as perfumery in cosmetics (Gospodinova and Krasteva, 2017; Bahadırlı, 2020; Thapa et al., 2020; Sukhikh et al., 2021). It has also been used for resisting gastric, paradontosis, diarrhoea and duodenal ulcers (Ivanova et al., 2005). The extract of *C. coggygria*, which is beneficial for eye ailments, is also used in a cholagogue fever (Li, 2009). In addition, the dried leaves and other parts of *C. coggygria* are used as antipyretics in traditional Chinese medicine (Thapa et al., 2020; Sukhikh et al., 2021). In addition, *C.*

*coggygria* syrup protects the liver and most organs from chemical damage, affects the bile flow rate, and prevents inflammation, tumour, microbial infections and increases the immune system (Milosavljevic et al., 2021).

Prior phytochemical analysis of *Cotinus coggygria* identified showed almost having the same chemical composition with monoterpenic hydrocarbons predominant (Novakovic et al., 2007; Milosevic et al., 2008; Fraternale and Ricci, 2014; Sukhikh et al., 2021).

There have been studies on leaf essential oil of the plant in Türkiye (Demirci et al., 2003; Bahadırlı, 2020). However, this is the first report, to our knowledge, on inflorescence chemical profile on were collected from the naturally growing plants on the Sarikamis region of Kars, Turkey.

## Materials and Methods

### Plant Material

The fresh aerial parts of *Cotinus coggygria* Scop. were collected from the naturally growing plants in the Sarikamis region of Kars, Turkey in 2015. The plant materials were collected air-dried under shade, carefully separated into flowers and leaves, and kept for further analyses of the taxonomic identification of the collected plant materials was done by a plant taxonomist from the Department of Biology, Yuzuncu Yil University, Van, Türkiye.

### Analysis of the Essential Oil

Drying 100 g of the plant sample in the Clevenger for 3 hours was subjected to hydrodistillation. The obtained oils were extracted with the help of distilled water and stored at 20 °C in an unopened bottle for later use. At the end of the analysis, the yields are based on the dry matter of the plant samples.

### Gas Chromatography-Mass Spectrometry Analysis

The plant materials analyses were carried out on Shimadzu QP2010 brand model gas chromatography quadrupole-mass spectrometry system fitted with a TRB-WAX column (30 m × 0.25 mm film with 0.25 µm thickness). The carrier gas was helium with a linear velocity of 40 cm sec-1, the split ratio was 1:50 at a flow rate of 1.2 mL min-1. Primarily, oven temperature 60°C for 3 min and then programmed to raise from 60 to 240°C at 9°C min-1 and at last held isothermally for 5 min at 240 °C. The pre-processing took a total of 33 minutes injection and the ion source temperature in the device used was 240 °C. The injection volume was 1 µL in the 1/50 split mode (Devi et al., 2021). Masses were afflicted at 70 eV. The measured mass range was between 40 and 300 m/z. The contents of essential oils were defined by equal related retention times and mass spectra with authentic samples from essential oil library data (NIST 27, Wiley, 7 and NIST 147) and by comparing relative rate indices (RI) with published data.

## Results and Discussion

The percentage of every identified compound in the leaf essential oil of *Cotinus coggygria* has given in Table 1, in which the ingredients are listed in accordance to with their retention indices. The GC/MS chromatogram of the essential oils has also appeared in Figure 1. The result analysis of a total of eleven compounds was characterized accounting for 99.96% of ingredients in the leaf oil samples. The total essential oil content for flower and leaf samples were 1.1% and 0.9%, respectively (Table 2).

The oil, characterized by the high amount of monoterpenes, was dominated by limonene (48.53%) and cis-ocimene (23.67%). The other major ingredients were cis-ocimene (7.22%), β- Phellandrene (5.62%) and α-pinene (3.72%). In the inflorescence oil, however, 21 ingredients were identified, representing 100% of the total oil. Thymol (31.33%) and carvone (20.57%) were found as the predominant ingredients. The essential oil was mainly characterized by sesquiterpene. The chromatogram profile of *C. coggygria* leaf oil has presented in Figure2.

As a result of some studies thirty-eight tables of contents from the category of monoterpenes and sesquiterpenes were described in the essential oils from the flowers of *Cotinus coggygria* from the South Serbia. In analysis, the main components in the essential oils were determined as monoterpene limonene (39.5%, 6.5% and 3.39%) and α-pinene (16.0, 15.1% and 21.9%) from leaves, flowers and stems has obtained (Milosevic et al., 2008).

## Conclusion

In a study conducted on the same plant, in laboratory studies of wild *C. coggygria* plant from two regions in Serbia, such as Deliblatska pescara and Zemun, it was determined that monoterpenic hydrocarbons were prominent as 87.4% and 93.1%, respectively. As a result of the analysis, the main components of both extractions were limonene (47.0% and 39.2%), (Z)-β-ocimene (16.4% and 26.3%), α-pinene (8.2% and 8.4%), (E)-β-Osimene (4.6%) and 9.0% and terpinolene (6.8% and 5.3%) were found to be the same (Novakovic et al., 2007). Oil samples from Bulgaria contain basic components such as 8.5% α-terpineol, 43.0% α-pinene, 5.7% cymene, 2.4% globulol 11.4% β-pinene (Thapa et al., 2020). The main components of Hungarian oil samples were a-pinene 24.4–34.3%, δ-3-carene 4.6–11.0%, limonene 30.0–40.0%, β-pinene 7.6–20.2% and α-terpinolene 3.3–10.6% (Hethelyi et al., 1986). Similarly study ingredients of *C. coggygria* show that was 6.9% β-ocimene, 7.6% α- pinene (Rezaee et al., 2019). The essential oil composition of the flowers and leaves of *Cotinus coggygria* from Greece has previously been studied revealing limonene (67.4% and 41.4%) as the major constituent of both leaf and flower oil, respectively, where α-pinene was reported as the second main ingredient of either leaf (14.7%) or flower (32.4%) (Tzakou et al., 2005). GC/MS analysis of volatile oil of flowering aerial parts smoke wood from Italy revealed α-pinene (8.5%), (Z)-β-ocimene (15.2%), limonene (47.1%) and (E)-β-ocimene (5.3%) as the predominant ingredients (Fraternale and Ricci, 2014). While, camphene (8%), myrcene (50%), linalool, α-pinene (10%), and α-terpineol were described as the primary ingredients in the French *Cotinus coggygria* oil (Gildemeister and Hoffmann, 1959). The most predominant ingredients from Turkish smoke wood were determined as (E)-β-ocimene (9.7 %), (Z)-β-ocimene (27.9 %) and limonene (48.5 %) (Demirci et al., 2003). The unique finding of the current study was identification of thymol as the major constituent of leaf essential oil, which hadn't been observed in any of the previous research. Moreover, characterization of phytol

from diterpenes chemical class could be considered as the other novelty of the studied oil.

Table 1. Ingredient of the essential oils from *C. coggygria* Scop. aerial part.

| Peak | Ingredient | RI | Flowers | Leaf |
|---|---|---|---|---|
| 1 | α-pinene | 908 | - | 3.72 |
| 2 | β-myrcene | 941 | - | 0.77 |
| 3 | Limonene | 955 | 10.56 | **48.53** |
| 4 | cis-ocimene | 969 | 6.40 | **23.67** |
| 5 | trans- β-ocimene | 976 | 1.61 | 5.62 |
| 6 | α- terpinolene | 990 | 0.84 | 2.75 |
| 7 | trans-caryophyllene | 1090 | 0.79 | 2.31 |
| 8 | trans-anethole | 1093 | - | 3.47 |
| 9 | α-amorphene | 1108 | 0.54 | - |
| 10 | α-terpinyl acetate | 1112 | 0.47 | - |
| 11 | β-bisabolene | 1120 | 1.33 | - |
| 12 | Geranyl acetate | 1128 | 0.37 | - |
| 13 | δ-cadinene | 1130 | 1.27 | - |
| 14 | Anethol | 1148 | 11.55 | - |
| 15 | trans-anethole | 1151 | 2.34 | - |
| 16 | Caryophyllene oxide | 1189 | 0.38 | 0.84 |
| 17 | Methyl eugenol | 1193 | 1.23 | - |
| 18 | Cedrenol | 1221 | 2.62 | - |
| 19 | Cedrol | 1224 | - | 7.19 |
| **20** | **Thymol** | 1232 | **31.33** | **-** |
| **21** | **Carvone** | 1239 | **20.57** | **-** |
| 22 | cis-asarone | 1242 | 0.94 | - |
| 23 | β-eudesmol | 1245 | 0.79 | - |
| 24 | Solvanol | 1274 | 1.58 | - |
| 25 | Phytol | 1326 | - | 1.09 |
| Total (%) | | | **100.00** | **99.96** |
| Essential oil content (%) | | | **1.1** | **0.9** |

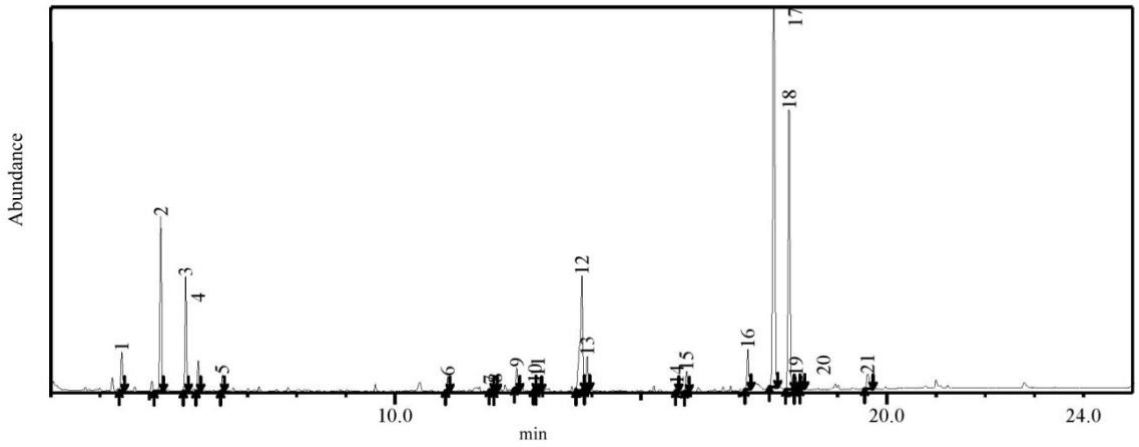


Figure 1. Chromatogram of *C. coggygria* Scop. flower.

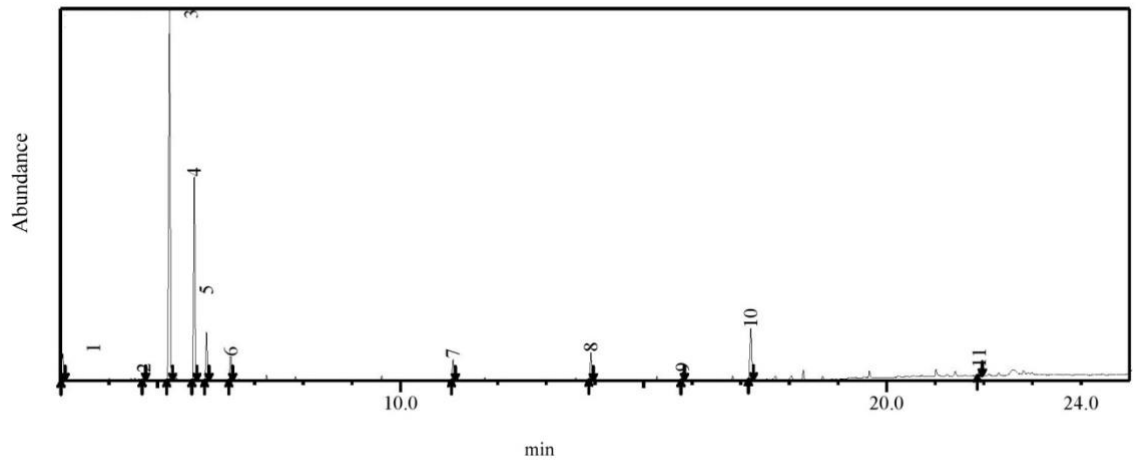


Figure 2. Chromatogram of *C. coggygria* Scop. leaf.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Ethics committee approval is not required.

### Funding

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Evaluation of genetic structure of pistachio through whole genome resequencing

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## Abstract

Pistachio (*Pistacia vera* L.) is the only edible and cultivated species. Pistachio is the only economically importance and dioecious species in the genus *Pistacia*. There are basic problems in pistachio breeding such as dioecious flower structure, long juvenile period and alternate bearing. These problems can be overcome not with classical breeding methods, but with modern molecular breeding methods. In this study, very high numbers of single nucleotide polymorphism (SNP), insertion/deletion (InDel), structural variants (SV) and copy number variation (CNV) were determined by using the next generation sequencing data of the pistachio genotype obtained with 15x sequencing coverage. A total of 1,785,235 SNP, 260,683 InDel, 5,227 SV and 1,914 CNV variants identified in PvF217 pistachio genotype. The variant density was calculated as one variant per 292 base. The distribution of the obtained variants to the Siirt reference genome was obtained. In addition, all variants were annotated to the reference genome and exonic and genomic variants were described using Annovar. These data will be used to consist of a molecular database in pistachio breeding for DNA fingerprinting, discovering unique cultivar specific alleles and to identify quantitative trait loci related to important nut traits.

## Keywords

Pistachio, Resequencing, Genome, SNP

## Introduction

Pistachio (*Pistacia vera* L.) takes place within the genus *Pistacia* and Anacardiaceae family. The genus *Pistacia* included at least 11 species (Kafkas, 2006) and pistachio is the only with economic value. *P. vera* is dioecious flower structure and it pollinates with wind with some exceptions (Kafkas et al. 2000). Its ploidy level is diploid and haploid chromosome number is n=15 (Kafkas, 2019). Pistachio has originated in central Asia, later spread from its origin to Mediterranean region of Southern Europe, Middle east, North Africa, China, United States and Australia (Kafkas, 2019).

The primary problems in pistachio breeding are long juvenile period, dioecious blooming nature and alternate bearing event (Gündeşli et al., 2019). The description of the traits and development of loci associated with traits in pistachio breeding are time-consuming processes such as the characterization of genetic resources and evaluation of segregation population. However, development of markers linked with pest and disease resistance, nut quality traits, alternate bearing and yield in pistachio were not performed any studies (Gündeşli et al., 2021). Overcoming these limitation factors in breeding programs can be only possible molecular approaches using next generation technologies (NGS) (Kafkas, 2019).

Although, simple sequence repeats (SSRs) markers have been utilized in different molecular characterization studies of different species such as apricot, apple, pear, pistachio and quince (Hormaza, 2002; Potts et al., 2012; Fan et al., 2013; Zaloglu et al., 2015; Guney et al., 2019), they are not adequate for marker assisted selection in breeding programs. Until now, different molecular marker systems have been improved and utilized in genetic characterization and mapping of pistachio (Kafkas et al., 2006; Motalebipour et al., 2016, Khodaiminjan et al., 2018; Karcı et al., 2020; Karcı et al., 2022 (unpublished)). However, only

several molecular markers based on SNP developed by (Kafkas et al., 2015, Khodaeiminjan et al., 2017) have been used efficiently for marker assisted selection (MAS) according to sexes in early stage of plant development in pistachio, to date (Kafkas et al., 2017).

Although, there are many genetic characterization studies based on different molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), and SSR in pistachio (Kafkas et al., 2006; Zaloglu et al., 2015; Motalebipour et al., 2016; Karcı et al., 2020), there is no markers associated with pest or disease resistance, yield and nut quality characters in pistachio exception of sex markers developed using RAD-seq technology (Kafkas et al., 2015). Recently, Kafkas et al. (2022) studied over the pistachio sex chromosome and they identified ZW sex choromosome using whole genome resequencing (WGR) technology. About 12.6 Mb W specific female genomic region were identified in chromosome 14. The researchers also represented that high chromosomal assembled of female Siirt cv. and male Bagyolu cv. genomes.

The main objectives of the current study, to consist of the workflow of the variant detection on the resequencing data and database of the pistachio such as SNP array for future revealing genes or QTL regions associated complex and important pistachio traits.

## Materials and Methods

### Plant Material and DNA Extraction

Pistachio genotype (PvF217) was used in this study and pistachio leaf sample was collected from Çukurova University, at the Research and Experimental area of Agriculture Faculty in Adana province of 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., 2006b). 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 ºC for further analysis.

### Resequencing and Variant Calling Analysis

The resequencing was performed in Illumina Hi-seq 2500 and genome sequencing coverage is 15x. The clean pair end (PE) reads were obtained once low quality and adaptors cleaned with Trimmomatic (Bolger et al., 2014). The clean reads obtained from each genotype will be mapped to the reference genome of Siirt cultivar with the Bowtie2 (Langmead and Salzberg, 2012) program mem option. After the clean reads are aligned to reference Siirt genome (Kafkas et al., 2022; unpublished) using SAMtools (Li et al., 2009) view option, duplicate reads were eliminated with Picard tools. SNP and Indel loci were determined using the GATK (Genome Analysis Toolkit) program HaplotypeCallerSpark option with hard filtering option (QD < 2.0, QUAL < 30.0, SOR > 3.0, FS > 60.0, MQRankSum < -12.5, ReadPosRankSum < -8.0) (McKenna et al., 2010). Structure variant and copy number variation analysis was performed using Delly program with call and cnv options (Tobias et al., 2012). The detected variants were annotated to Siirt genome gff3 file for getting the information of variants using the Annovar program (Wang and Hakonarson, 2010).

## Results and Discussion

### Mapping and removing PCR duplicates

Approximately 12,5 Gb raw PE reads were generated from PvF217 pistachio genotype in Illumina using Hi-seq 2500. A total of 279,718 (0.34%) low quality reads and 5,588,708 (6.72%) polluted reads were removed and a total of 11,578,693,200 clean reads were obtained with 92.85%. A total of 77,102,620 of 77,191,288 clean reads were mapped to reference Siirt genome and the mapping rate was computed as 99.89 % in samtools flagstat option. The mapped reads were filtered according to mapping quality rate (MQ=30) and the rest of the reads were detected as 54,525,620 reads.

The remaining mapped reads were still PCR duplicates that reasons to detect false positive variants on the genomes. Thus, Picard tools marked the 5,537,304 (7.18%) PCR duplicates. As a result, a total of 48,988,316 mapped reads were ready for variant detection (Table 1).

Table 1. The raw, clean, low-quality, polluted, mapped, duplicates and remaining reads numbers and percentage of pistachio genotype

| Reads | Numbers |
|---|---|
| Raw Bases Number | 12,470,888,100 |
| Clean Bases Number | 11,578,693,200 |
| Low-quality Reads Number | 279,718 |
| Low-quality Reads Rate(%) | 0.34 |
| Adapter Polluted Reads Number | 5,588,708 |
| Adapter Polluted Reads Rate(%) | 6.72 |
| Mapped Reads Number | 77,102,620 |
| Mapped Reads Rate (%) | 99.89 |
| Remaining Reads Number | 54,525,620 |
| Remaining Reads Rate (%) | 70.64 |
| Duplicates | 5,537,304 |
| Duplicates Rate (%) | 99.08 |
| Remaining Reads Number | 48,988,316 |
| Remaining Reads Rate (%) | 876.56 |

### Detection of variants and distribution of the variants through the pistachio genome

Variant detection was carried out using two different variant calling program in linux terminal. Firstly, SNP, InDel were detected using GATK, SV and CNV bam files were utilized for detection variants. A total of 1,785,235 SNP and 260,683 InDel were mined in bam format file of PvF217 pistachio genotype using HaplotypeCallerSpark command in linux bash script. The distribution of the SNP and InDel variants were identified according to chromosomes and scaffolds of the pistachio genotype. Although, the most abundance SNP variants were detected on chr13, the least chromosome density in SNP loci was on chr2. The chromosome 13 has the most abundance InDel variants with 22,250 loci, and the scaffolds have the least resolution InDel loci (Table 2, Figure 1).

The SV and CNV variants were calculated as 5,227 and 1,914, respectively. The scaffolds and chr13 were identified to have high density genomic regions in both SV and CNV loci, while the least abundance variants were located on chr2 (Table 2, Figure 1).

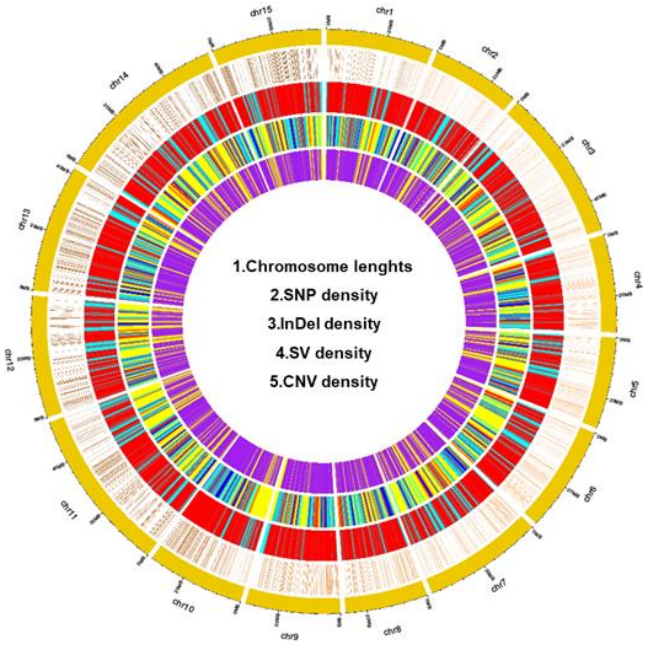


Figure 1. The chromosome lengths, SNP, InDel, SV and CNV variants (from outside to inside) distribution of the pistachio PvF217 genotype

The SV variants were classified according to types such as deletion (DEL), duplication (DUP), insertion (INS), invertion (INV) and translocation (TRA) and the number of these detected loci were calculated as 2,908, 595, 247, 418 and 1,059, respectively (Figure 2).

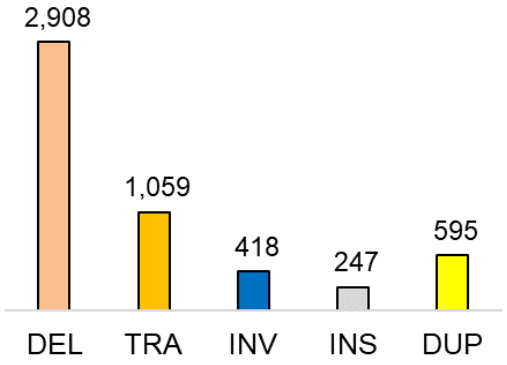


Figure 2. The types of structural variants of the PvF217 pistachio genotype

### Genome annotation of variants

The genome annotation was performed using Perl script in Annovar program. Totally, 1,785,235 SNP loci annotated to Siirt reference genome (Kafkas et al., 2022). The most variants were identified on intergenic genomic regions and the number of these variants were computed as 1,335,662. Similarly, the most abundance variants in InDel, SV and CNV were detected as intergenic variants 172,415, 3,103 and 1,511, respectively (Table 3).

Although a total of 73,957 SNP variants were determined on the genic regions, only 4,987 InDel variants were found in genic regions such as frameshift, nonframeshift, stopgain, stoploss (Figure 3).

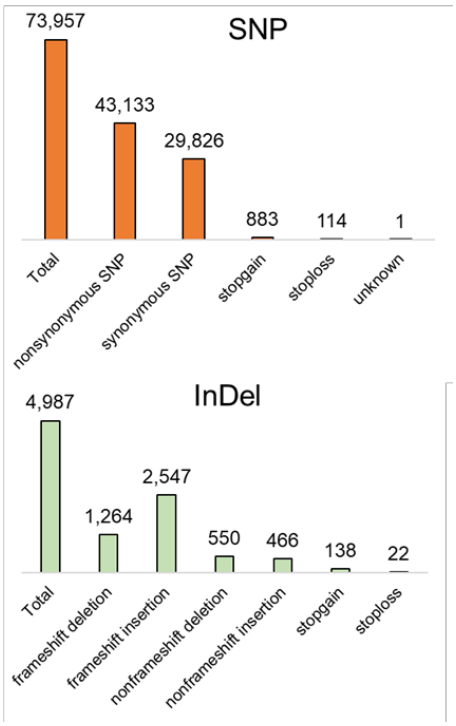


Figure 3. The number of the exonic SNP and InDel variants in detected of whole genome of PvF217

Table 2. The SNP, InDel, SV and CNV variants distribution of the PvF217 whole genome

| Chromosomes | SNP | InDel | SV | CNV |
|---|---|---|---|---|
| chr1 | 132,979 | 20,555 | 300 | 65 |
| chr2 | 76,409 | 12,442 | 194 | 48 |
| chr3 | 131,382 | 20,136 | 292 | 121 |
| chr4 | 113,709 | 16,355 | 260 | 80 |
| chr5 | 90,033 | 13,618 | 207 | 74 |
| chr6 | 96,404 | 15,427 | 235 | 81 |
| chr7 | 126,309 | 16,848 | 357 | 105 |
| chr8 | 96,685 | 14,900 | 353 | 66 |
| chr9 | 100,351 | 15,927 | 254 | 79 |
| chr10 | 103,469 | 16,377 | 332 | 92 |
| chr11 | 129,906 | 18,754 | 327 | 100 |
| chr12 | 116,307 | 14,831 | 309 | 130 |
| chr13 | 179,803 | 22,250 | 574 | 180 |
| chr14 | 107,718 | 16,322 | 298 | 104 |
| chr15 | 99,599 | 14,043 | 350 | 133 |
| scaffold | 84,172 | 11,898 | 585 | 456 |
| **Total** | **1,785,235** | **260,683** | **5,227** | **1,914** |

Table 3. The pistachio genotype (PvF217) genome annotation results belonging to SNP, InDel, SV and CNV variants

| Genomic regions | SNP | InDel | SV | CNV |
|---|---|---|---|---|
| UTR5 | 8,136 | 2,770 | 34 | 4 |
| UTR3 | 11,559 | 2,845 | 21 | 10 |
| UTR5;UTR3 | 20 | 9 | 1 | 0 |
| exonic | 73,957 | 4,987 | 911 | 59 |
| splicing | 571 | 202 | 11 | 2 |
| exonic;splicing | 3 | 1 | 0 | 0 |
| upstream | 91,591 | 20,337 | 337 | 100 |
| downstream | 82,569 | 17,164 | 323 | 86 |
| upstream;downstream | 11,356 | 2,728 | 54 | 8 |
| intronic | 169,788 | 37,221 | 432 | 134 |
| intergenic | 1,335,662 | 172,415 | 3,103 | 1,511 |
| ncRNA_exonic | 23 | 4 | 0 | 0 |
| **Total** | **1,785,235** | **260,683** | **5,227** | **1,914** |

The loci detected on the genic chromosome play important roles in the construction of the phenotypes of pistachio. The obtained results demonstrated that these variants can be associated with complex and governing from polygenes traits in pistachio.

There are some the limitation factors of the pistachio breeding such as dioecious character, long juvenile period and alternate bearing (Gündeşli, 2020a,b). The construction of the pistachio germplasm database is required in order to encountered like these breeding problems. However, preference of sequencing platforms is very important for large scale database in pistachio. Because, restriction-site associated DNA sequencing (RAD-seq), diversity arrays technology sequencing (DarT-seq) and GBS (Genotyping by Sequencing) utilize the restriction enzymes and the variants were identified by comparing restricted genome fragments between individuals. The cut genomic fragments cannot be used in other studies. However, resequencing NGS data can be used in order to generate SNP array for genome wide association studies and understood more deeply the complex agronomical important traits in pistachio. Recently, many resequencing findings reported in cucumber (Liu at al., 2021), camelina (Li et al., 2021) and hemp (Ren et al., 2021). On the other hand, the sex regions can be determined using resequencing data in QTL-seq that rapid identification of the sex regions in guinea (Tamiru et al., 2017).

To date, there are no studies related with development of the markers for marker assisted selections in pistachio breeding exception of sex markers developed by Kafkas et al., (2015) and Khodaieminjan et al., (2017). Thus, the obtained results and NGS data in this study can be used for future marker assisted breeding programs in order to develop unique and rare alleles for cultivar fingerprinting; detect the markers associated with nut quality traits, pest and disease resistance, phenological traits, morphological traits in pistachio.

## Conclusions

In the present study, 15x resequencing data of pistachio genotype were analyzed in linux terminal using different variant calling program. A robust circus plot was produced and distribution of the SNP, InDel, SV and CNV variants were illustrated in through whole genome pistachio. A total of 2,053,059 variants were detected and 79,914 variants were identified in exonic regions. The resequencing data allowed the allelic variations that can be applied for identifying genes useful to pistachio breeding programs. The consisted of the workflow variants detection can be applied for other cultivars and genotypes. The presented data will be useful in cultivar fingerprinting, germplasm characterization, phylogenetic studies, association and QTL mapping studies in pistachio.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# An evaluation on the current state of water buffalo breeding in Diyarbakır, identification of problems and solutions

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## Abstract
The present study aimed to determine the current state of water buffalo breeders in Silvan, Bağlar, Yenişehir, and Sur counties of Diyarbakır province in Turkey, and to reveal the aspects of shelter, grazing, calf breeding, brood use, meat and milk production practices, care and health protection, expectations from breeding practices and current problems. The results of the face-to-face surveys were used carried out with a total of 147 business owners engaged in water buffalo breeding in four counties. In the research, the average number of water buffaloes per farm was determined to be 11.03. The breeders have reported that they continue to raise water buffaloes for reasons such as making a living, high quality of water buffalo milk, and benefiting from the available subsidies. The average lactation period of water buffaloes was 7.1 months, the average daily milk obtained per animal was determined to be 3.63 liters. The milk obtained is mostly evaluated as yogurt, cheese, and butter. As a result, it was determined that almost all of the breeders are satisfied with water buffalo breeding, they see breeding as a family profession, and they are aware of the quality and importance of water buffalo products.

## Keywords
Anatolian Water Buffalo, Cattle, Diyarbakir, Breeding

## Introduction
Water buffalo is a species that has economic value unique to the region in many parts of the world. Quality milk and dairy products add a special value to the water buffalo. Water buffalo is highly resistant to natural conditions and diseases, has a high feed conversion ability, can convert poor quality roughage into meat and milk, which is very important in human nutrition, and its breeding costs are lower than cattle breeding, comprising the important advantages of water buffalo breeding (Canbolat, 2012).

Water buffalo is much more suitable especially for pasture livestock, as it does not choose feed and has the high conversion ability for roughage better than other ruminants. Water buffalo can make good use of the diseased pastures that cattle and sheep cannot benefit from. They can easily adapt to their environment and enjoy rolling in wet and muddy areas (Williamson and Payne, 1968; Fisher, 1975).

Water buffalo milk has high values especially in terms of protein, fat, and lactose ratio compared to other milk-producing species. The high-fat content makes it more preferred in making yogurt, cheese, and butter. It is sold at higher prices compared to cow's milk due to its better efficiency and taste in cheese and yogurt production. In Turkey, products such as lüle kaymağı (a traditional cream), Afyon cream, confectioneries, dairy desserts, ice cream, and butter are produced from water buffalo milk (Atasever and Erdem, 2008). The famous Italian Mozzarella cheese is obtained from water buffalo milk (Uslu 1970, İlaslan et al. 1983).

Carcass yield in water buffaloes is lower than that in cattle (Akdağ, 2004). However, water buffaloes have important advantages such as being able to benefit from

low-quality roughage and high feed conversion ratios, high resistance to difficult climatic conditions and diseases, and finding buyers for their products at higher prices. The unique structures of meat and milk obtained from water buffalo (meat contains low fat and cholesterol, milk contains high fat) give intense consistency and flavour to foods such as sausage, cheese, yogurt, and cream. However, in Turkey, the potential for organic breeding of water buffalo meat and milk and geographical is very high. Due to its thickness, water buffalo leather is in the industry as bag and furniture leather (Özkan et al., 2017).

The water buffaloes that exist and are cultivated in Turkey have originated from the Mediterranean water buffalo, a subgroup of the river buffalo, and are also called the Anatolian Buffalo (Soysal et al., 2005).

Translated as "water cattle" in Turkish, water buffalo is also called by different names such as Camız, Camış, Donbey, Kömüş, and Gameş depending on the regions where it is raised (Sarıözkan, 2011; Tatar, 2020).

India, Pakistan, China, Nepal, Egypt, Myanmar, the Philippines, and Vietnam are listed as the leading countries in terms of water buffalo presence in the world. While India accounts for 53.76% of the world's current water buffalo stock, it accounts for 86.7% of the water buffalo stock raised together with Pakistan and China (FAOSTAT, 2020).

Water buffalo breeding has attracted a lot of attention in Turkey in recent years. In addition to the subsidies and incentives given to water buffalo breeding, the number of water buffaloes in Turkey has been increasing gradually, with the demand of consumers for products obtained from water buffalo milk relatively increasing. With the 'Anatolian Buffalo Breeding Project in the Hands of the Society', established in Turkey in 2009, a regular increase has been observed in the number of water buffaloes since 2010 (Turkstat, 2021).

Samsun in the North Anatolian Region, Kayseri in the Central Anatolian, Tokat in the northern coastal areas, İstanbul and Balıkesir in the Marmara Region, Bitlis, Muş and Sivas in the East Anatolian Region, Diyarbakır in the Southeast Anatolian Region and Afyonkarahisar in the Aegean Region are the provinces where water buffalo breeding is carried out intensively in Turkey (Turkstat, 2021).

According to the TURKSTAT 2021 data, there are a total of 185,574 water buffaloes in Turkey. Samsun is the province with the highest number of water buffaloes in Turkey with 23 633 animals. Samsun is followed by Diyarbakır with 15 914 animals, Istanbul with 15 598 animals, Tokat with 10 846 animals, Bitlis with 10 123 animals, and Muş with 7 591 animals (Table 1).

Table 1. Water buffalo assets by Province in Turkey

| Province | Water buffalo asset |
|---|---|
| Samsun | 23633 |
| Diyarbakır | 15914 |
| İstanbul | 15598 |
| Tokat | 10846 |
| Bitlis | 10123 |
| Muş | 7591 |
| Kayseri | 7528 |
| Afyonkarahisar | 7137 |
| Balıkesir | 5475 |
| Sivas | 5363 |

The present study aimed to make an evaluation on the general characteristics, shelter conditions, grazing, and shepherd use, herd management, calf breeding methods, brood use, meat and milk production practices, health protection and expectations and problems of breeders in the counties of Diyarbakır, where the water buffalo asset is intense.

## Materials and Methods

In the present study, data obtained from face-to-face surveys with a total of 147 business owners in 19 villages of Bağlar, Yenişehir, Sur, and Silvan counties, where water buffalo breeding is intense, were used. The surveyed businesses were selected from those that are not members of the Anatolian Buffalo Breeding Project in the Hands of the Society. The survey applied to the breeders consisted of a total of 121 questions.

Table 2. Counties surveyed and the number of surveys conducted

| County | The Number of Surveys |
|---|---|
| Bağlar | 42 |
| Sur | 20 |
| Silvan | 69 |
| Yenişehir | 16 |
| Total | 147 |

Table 3. The number of water buffaloes owned by the surveyed counties and businesses

| County | Silvan | Sur | Yenişehir | Bağlar |
|---|---|---|---|---|
| Total (n) | 5107 | 1988 | 1710 | 1600 |
| Business (N) | 717 | 116 | 171 | 606 |

The water buffalo assets of the districts where the research was conducted constituted 62.2% of the Diyarbakır water buffalo asset. According to the districts of Bağlar, Silvan, Sur, and Yenişehir, the water buffalo assets in the surveyed businesses were 14.0%, 5.8%, 10.0%, and 37.9%, respectively. In other words, although it varied from district to district, it was seen that the surveyed districts constituted 15.5% of the total water buffalo existence. Considering that the study was carried out in businesses outside the scope of the "Anatolian Buffalo Breeding Project in the Hands of the Community", this ratio was substantial.

The survey was carried out in the form of filling out the questionnaire with face-to-face interviews with the breeders between August and October 2018.

The survey questions were handled under seven main titles regarding water buffalo breeding.

1. Information on Breeders and Businesses
2. Shelter Status
3. Information on Utilization of Pasture and Shepherd Use
4. Calf Raising and Brood Use
5. Meat and Milk Production Practices
6. Care and Health Protection Practices
7. Expectations and Problems of Breeders

The IBM SPSS statistical 24.0 package program was used in the evaluation of the data. The obtained data were first entered into the Excel package program. Then, descriptive statistics, frequencies, and percentage values were used in the evaluation of the data.

## Results and Discussion

### Information on the Farmers and Enterprises

In the present study, 92.5% of the breeders participating in the survey were male while 7.5% were female. It was determined that 57.1% of the breeders were primary school graduates, 21.8% were secondary school graduates, 15% were high school graduates and 2.7% were college graduates. It was determined that 3.4% of the breeders participating in the survey were illiterate. The fact that the active population, which is the source of the workforce, was between the ages of 15 and 64 was also an indication that the family workforce potential is quite high in the businesses studied. Yılmaz (2013) have reported that water buffalo breeders were mostly between the ages of 41-50 and that there were not many breeders older than 60 years and younger than 30 years old. Kaptan (2019) has stated that breeders were generally over 61 years old and that the number of breeders under 30 years of age was very low.

The average number of individuals per business was determined to be 8.73. This value was considerably higher than the household average of Turkey of 3.4 and the household average of Diyarbakir of 4.97. Işık (2015) have reported the average population per business as 6.68 while Yılmaz (2013) determined the average population per business to be 11.

It was determined that the breeders participating in the survey have been breeding water buffalo for an average of 18.18 years. It was determined that 11.5% of the farmers have been carrying out breeding for five years or less, 15% of the farmers have been breeding for 6-13 years, and 73.5% of the farmers have been breeding for 13 years or more. This ratio is an indication that water buffalo breeding is a family profession. Özger (2018) determined the active breeding period of the breeders to be 26 years.

Of the breeders participating in the survey, 69% stated that they do cattle breeding with water buffaloes. The ratio of those who breed sheep with water buffalo was 13% and the ratio of those who breed goat with water buffalo was 5%. The ratio of businesses engaged in sole water buffalo breeding was determined to be 13%. It was determined that 22% of the breeders did not own the land. While the land assets between 25 and 50 decares were 16%, those between 51 and 100 decares were 23%, those between 101 and 200 decares were 11%, those between 201 and 400 decares were 24%, and the ratio of businesses with 400 decares or more was 4%. The average land assets of businesses that own the lands were calculated as 174.9 decares. While the average dryland per business was 133.4 decares, the irrigated land ratio was 136.35 decares. Işık (2015) has reported the average land size per farm as 26.26 decares while Avcı (2015) as 144.8 decares. The average land size obtained in the present study was found to be higher than the specified values.

While 67.2% of the breeders participating in the survey had 10 or fewer water buffaloes, the average number of water buffaloes per farm was determined to be 11.03. Also, it was determined that they have an average of 6.36 water buffaloes, 2.36 bulls, 2.35 male calves, and 2.22 female calves per farm. In their study, Çiftçi and Yılmaz (2019) determined the average number of water buffaloes per farm as 8.84. Işık (2015) has reported that the number of water buffaloes per farm was 10.11, an average of 1.29 male water buffaloes, 4.95 female water buffaloes, 1.69 male calves, and 2.18 female calves per farm. Yıldız et al. (2021) have reported that the average water buffalo assets per farm were 9.33.

### Shelter Status of Businesses

All the breeders have stated that they raised their water buffaloes in their barns in the village. While 32% of the breeders only kept water buffalo in their barns, 68% reported that they kept water buffalo and cattle together.

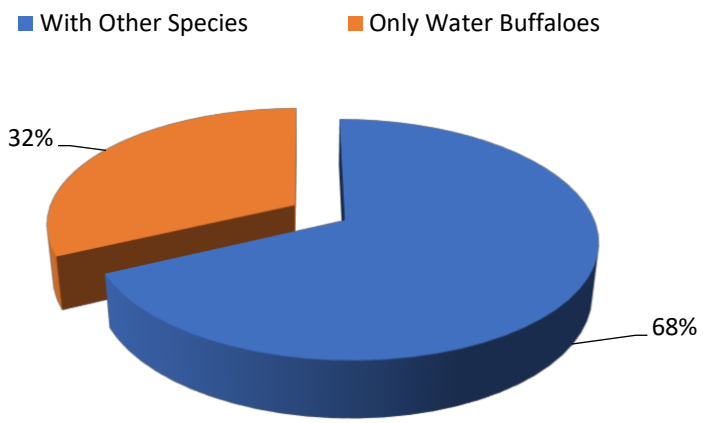


Figure 1. How animals benefit from shelters in businesses

It was determined that 35% of the barns in the businesses were located under the houses of the breeders, while 24% were adjacent to the houses and 41% were independent of the houses. Altınbaş (2003) has stated that 52% of the shelters were under the breeders' houses, 44% were independent of their houses and 4% were adjacent to their houses. It was determined that all the independent shelters had closed tied stalls. In 82% of the barns, the floor consisted of reinforced concrete, while in18% consisted of soil. Avcı (2015) have reported that all of the breeders had a concrete barn floor and emphasized that even if the concrete coating increases the cost of the building, it is important in terms of hygiene. Similarly, Altınbaş (2003) have reported that all the barn floors of the businesses were reinforced concrete.

While 71.4% of the breeders preferred brick-briquette as the wall material in their businesses, 17% preferred adobe, 10.89% stone, and 0.71% wood material. The ratio of those using tiles as the shelter roofing material was 73%, the ratio of those using wood was 9% and the ratio of those using soil was determined to be 18%. Altınbaş (2003) determined that 34.6% of the shelter roofing materials of the farms are made of tiles, 28.4% of wood, 1.2% of soil, and 35.8% of material obtained from zinc. Avcı (2015) reported in his study that wooden materials were used in all the shelter roofs of the farms owned by the breeders.

One of the most important problems of closed shelters is ventilation. In the research, 80% of the enterprises do not have ventilation shafts. It was determined that there are an average of 1.64 ventilation shafts in the shelters with ventilation shafts. While the ratio of the number of farms with 5 or less than 5 windows in the barns of the breeders was 89.8%, the ratio of the farms with 5 or more windows was 14.3%. The number of windows per establishment was calculated as 3.2. Since the type of windows is not suitable for measuring, their measurements could not be taken. Altınbaş (2003) stated that the ratio of businesses with ventilation shafts was 13.8% and the average number of chimneys per business was 1.64.

Of the breeders, 10.2% provided water for their water buffaloes only from the fountains in the village, 11.6% from fountains and streams, 34.7% from fountains and rivers, 9.5% from fountains and wells, 1.4% from rivers and wells, and 32.7% have stated that they benefited from fountains, rivers, and wells for their water needs. Altınbaş (2003), in the survey conducted with water buffalo breeders in Bartın province, has reported that the businesses benefited from the fountains in their courtyards as the source of water.

Of the breeders who participated in the survey, 68.7% stated that they preferred plastic containers, 24.5% metal, and 6.8% concrete containers. Altınbaş (2003) stated in his study that 7.5% of the breeders prefer metal buckets, 2.5% wooden buckets, 1.3% concrete, and 88.7% plastic buckets. It was determined that the breeders preferred 73.5% concrete, 12.9% metal, 8.8% plastic, and 4.8% wooden feeders as the material.

### Information on Utilization of Pasture and Shepherd Use

In the study, all of the breeders declared that they benefited from the village common pasture for feeding their water buffaloes. While 97.3% of the breeders who participated in the survey have reported that they grazed their water buffaloes in the pasture with other animal species, 2.7% of the breeders have reported that they took their water buffaloes out as a single herd in the pasture. Altınbaş (2003) has reported that the ratio of the breeders who do not graze their water buffaloes with other species was 35%, and the ratio of those who graze with other species was 65%. In the districts where the present survey was conducted, the annual average duration for the water buffaloes for pasture utilization was calculated as 10.36 months. Altınbaş (2003), in the study conducted in the province of Bartın, determined that the water buffaloes in the region benefited from the pastures for 6.6 months. In their study, Çiftçi and Yılmaz (2020) determined the annual average duration for the breeders for rangeland utilization as 1-2 months, 3-6 months, and 7-8 months, and determined the utilization rates from the pasture as 0.00%, 66.91%, and 33.09%, respectively. Kaptan (2019) has reported that 42% of the breeders benefited from the pasture for 7-8

months, and 55% for 9 months or more. The same researcher has also reported that more participation was achieved in the 3-6 months rangeland utilization period in their research and that they had at least 6 months or more pasture utilization period.

In this study, 89.1% of the breeders have stated that they preferred to use a shepherd for their water buffalo care and pasture grazing needs whereas 10.9% have stated that they did not find it necessary to use a shepherd. It was determined that 23.7% of the farms where shepherds were used, one of the family members performed the shepherd's job whereas in 76.3% they employed the shepherd outside the family members.

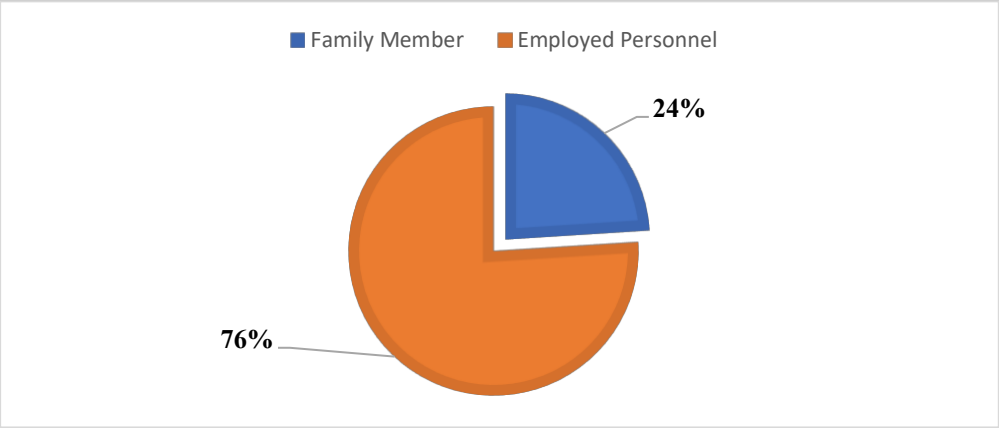


Figure 2. The shepherd employment status in the businesses

While 34.3% of the breeders who participated in the survey have stated that the number of shepherds used in the businesses was 1, 29.8% have stated that the number of shepherds was 2, and 35.9% have stated that the number of shepherds used in the businesses was 3.

## Breeding and Milking

The reproductive cycle in water buffaloes is different from that in cattle. The answers received from the questions asked to the breeders on this subject were that 87.8% of the breeders stated that the gestational period of their water buffaloes was 10 months while 10.2% stated 10.5 months and 2% stated 11 months. Altınbaş (2003) reported the gestational period of water buffaloes as 323.5 days (approximately 11 months) according to the answers received from the breeders in the survey carried out with water buffalo breeders in Bartın. Şekerden (2001) has stated that the average gestational period in water buffaloes was 315 days (308-321 days).

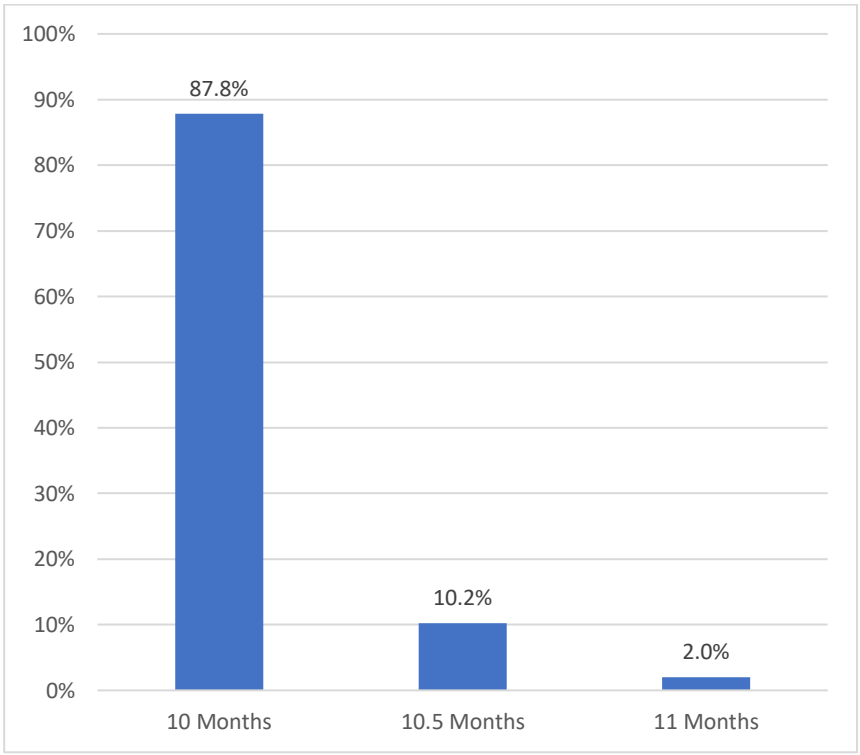


Figure 3. Responses regarding the gestational period of water buffaloes

In terms of umbilical cord care, one of the first interventions to the pup after birth, it was determined that 13.6% of the breeders cut the umbilical cord of the calves whereas 86.4% did not cut the umbilical cord after birth. It was determined that the milking performed in all the businesses participating in the survey was carried out by the women in the family. Hand milking was carried out in all the businesses. Özger (2018), in their study on "the Economic Analysis of water buffalo Breeding Activity" in Iğdır province, has stated that 94.6% of the animals were hand milked in the businesses surveyed. Kaptan (2019) has stated that 94% of the breeders surveyed reported that milking was done by their spouses, 3% by their children, and 3% by the workers employed.

The mean weaning age of calves was determined to be 4.96 months, the earliest weaning age was 1 month, and the latest weaning age was 12 months. In the present study, it was found that 22.4% of the breeders wean their calves in 3 months or less than 3 months, 68.7% wean their calves between 4-6 months, and 8.9% wean their calves in 7 months or more. Altınbaş (2003) determined that the average age of weaning of calves in businesses was 4.9 months. This result was similar to the data obtained in the present study.

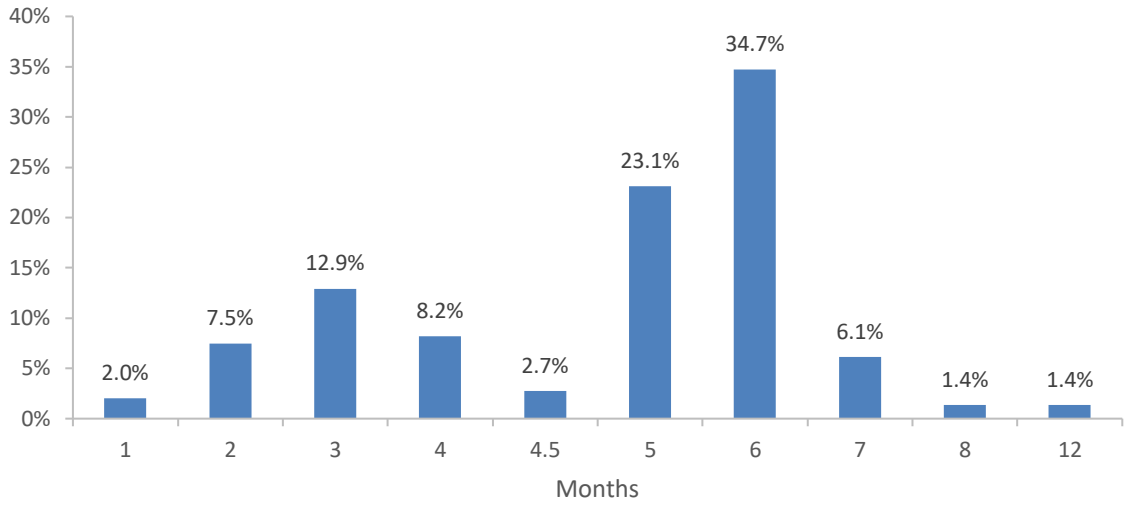


Figure 4. Weaning times in calves

It was determined that the period of feeding the calves for the first time after birth was between 3 days and 3 months in the surveyed businesses. Since the milk yield of water buffalo cows is low, calves drink milk for varying periods depending on the farm. Of the breeders included in the survey, 20.4% have reported that they started full milking of water buffaloes 3 days after birth, 62.6% 1 week after birth, and 17% 1 month after birth. Altınbaş (2003) has reported that the calves were fed for the first time 43 days after birth.

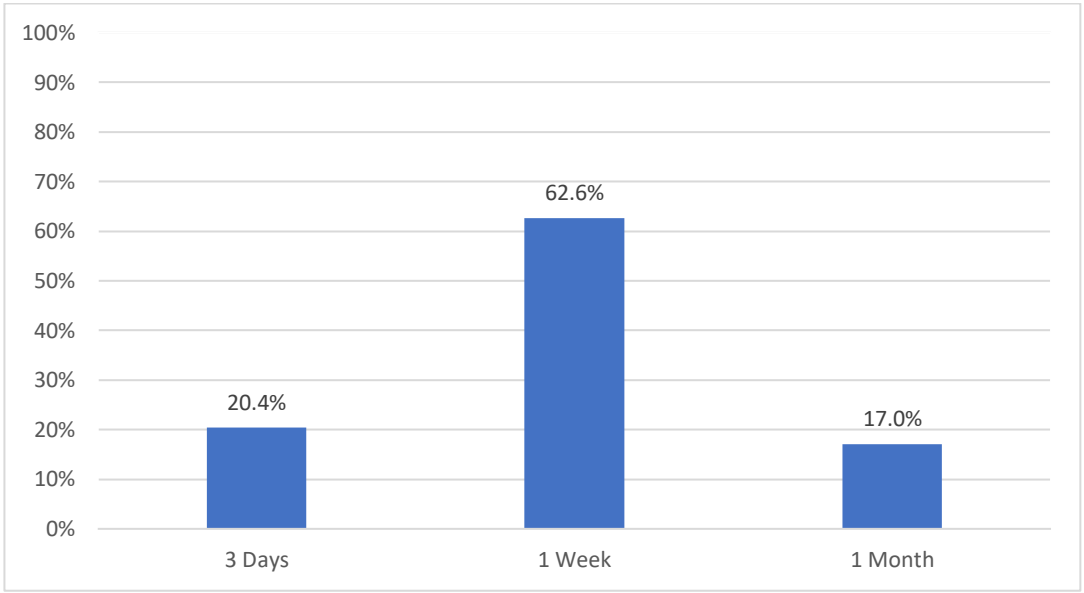


Figure 5. The onset of full milking in businesses

Of the breeders, 17% have stated that the lactation period of their water buffaloes was 6 months, 50.3% 7 months, 28.6% 8 months, and 4.1% 9 months during a lactation period. The mean lactation period was calculated as 7.1 months. Altınbaş (2003) determined the average lactation period in businesses as 6.3 months. Kaptan (2019) has reported that 64% of the breeders surveyed stated that the lactation period was 180-200 days while 36% stated 210-270 days.

In the present study, during the lactation period, 5.4% of the breeders stated that they carry out milking once a day whereas 94.6% stated that they applied two milkings a day. Bayram (2016), in their study in Samsun, determined that 69.2% of the farms milked once a day whereas 30.8% milked twice a day.

While 84.4% of the breeders have stated that they cleaned the udder before or after milking, 15.6% have stated that they did not clean the udder. Of the breeders that clean the udder, it was determined that 84.7% of the breeders cleaned the udder before milking, 2.4% after milking, and 12.9% both before and after milking. Özger (2018) has stated that 96.7% of the breeders carry out udder cleaning whereas 3.3% do not. Of the breeders, 26.5% have reported that they did not feed during milking whereas 73.5% have reported that feed during milking.

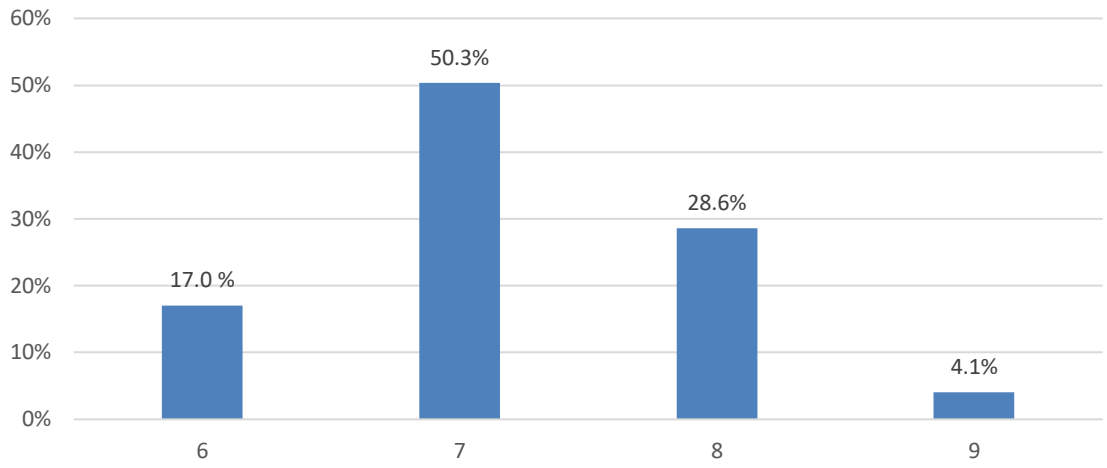


Figure 6. Lactation duration of water buffaloes

Of the breeders participating in the survey, 17.7% have reported that they used female water buffaloes for the first time at the age of 18 months, 48.3% at 24 months, 17.7% at 30 months, and 16.3% at 36 months for breeding. Regarding the age to use males for breeding for the first time, 38.1% of the breeders have stated 18 months, 53.7% 24 months, 2.7% 30 months, and 5.4% 36 months.

Since the Anatolian buffalo is a late-developing breed, the breeding age also occurs later. While the young water buffalo's participation in the herd is two years old for females, it reaches 18 months for male water buffaloes. In the present study, the average breeder selection age of the owners was calculated as 1.9 years. It was determined that 32% of the breeders set the brood selection age as 1.5 years, 59.9% as 2 years, 2.7% as 2.5 years, and 5.4% as 3 years. Altınbaş (2003) found the average age for breeding water buffaloes to be 24.3 months for males and females. Çiftçi and Yılmaz (2019) have reported that 27.21% of the breeders stated the average age for breeding water buffaloes as 18-22 months, 30.15% as 23-24 months, and 42.65% as longer than 25 months. In the present study, it was determined that 83% of the breeders met their breeding animal needs from their herd, while 17% meet their breeding animal needs from outside. Altınbaş (2003) has reported that 5% of the breeders supplied their breeding animals from their herds, 42.5% from outside, and 52.2% from both outside and their herds. Regarding the question, "What are the important selection criteria for male breeder selection?", 87.7% of the breeders listed the physical appearance, 93.9% the height at the withers, 36.1% color, 21.8% shank thickness, 7.5% rump width, and 1.4% suitable animal prices. Altınbaş (2003) determined that the breeders' male brood selection was made by looking at their body structure, horn, nose, leg, tail structure, short tail, and neck structures. Regarding the question, 'What are the important selection criteria in the selection of female broods?', 66% of the breeders listed the physical appearance, 21.8% height at the withers, 40.1% color, 6.1% shank thickness, 2.7% rump width, 32.7% milk yield of the animal's mother, 81.6% the animal's mammary gland size and 1.4% as the state of having horns. Altınbaş (2003) determined that female brood selection has been made by examining udder, horns, body structure, long tail, and ear structures. Of the breeders, 15% have stated that they feed their non-brood female and male animals for fattening, 95.5% have stated that they sold them to breeders, 2% have stated that they fed them as sacrificial, and 15% have stated that they sold them as broods. Altınbaş (2003), in the survey conducted with water buffalo breeders in Bartın province, has reported that 40.3% of the breeders feed their non-breeding female and male animals for fattening, 27.3% sell them to the breeder, 1.3% have stated that they fed them as sacrificial, and 31.1% sell them as broods. In the study conducted in Afyonkarahisar, Yılmaz (2013) determined that 47% of the breeders sell their brood water buffaloes after weaning, 37% slaughter immediately, 20% breed and feed them until they die, and 10% breed them for a few years and then slaughter them.

### Meat and Milk Production

Of the breeders, 60% have stated that they did not pay attention to the onset of the fattening time of their water buffaloes, 15% of the breeders have stated that they worked with animals aged 12 months, 10% 18 months, and 5% 24 months old. Of the breeders, 40% have reported that they fatten water buffaloes with a live weight of 200-300 kg. While 55% of the breeders pay attention to the fattening period, 40% of the breeders who pay attention determined the fattening period as 4-6 months, and 60% determine the fattening period between 8 and 10 months. Of the breeders, 46% have stated that they ended the fattening at 350-400 kg body weight, 38% at 450-500 kg body weight, and 16% at 600-650 kg body weight. Altınbaş (2003) has stated that the average age of the onset of fattening was 2.5 years, the weight of the water buffaloes was 250 kg per fattening, the average fattening period was 6 months, and the weight at the end of fattening was 400 kg.

While the ratio of those who sold the animals, they fattened at the end of the fattening was 85%, the ratio of those who stated that they consumed the meat of the slaughtered animals themselves was 15%. All the breeders have stated that they did not evaluate the skin and horns obtained from the animals after slaughter. The average milk yield per animal in the farms was determined to be 3.63 liters/day. While the milk volume taken from an animal in businesses was 2 L minimum, the highest milk yield was 6 L. Altınbaş (2003) determined the average daily milk yield per water buffalo during the lactation period in businesses to be 4.13 kg.

Examining Table 4 is examined, it is seen that 73.4% of the businesses produce 4 L or less milk per animal per day, while 26.5% produce more than 4 L of milk.

Table 4. Daily milk yield of water buffaloes in farms (on the date of the survey was held)

| Milk Yield | Frequency | Percentile (%) |
|---|---|---|
| 2.0 | 8 | 5.4 |
| 2.5 | 12 | 8.2 |
| 3.0 | 52 | 35.4 |
| 3.5 | 3 | 2.0 |
| 4.0 | 33 | 22.4 |
| 4.5 | 13 | 8.8 |
| 5.0 | 24 | 16.3 |
| 6.0 | 2 | 1.4 |
| Total | 147 | 100.0 |

It was determined that 96.6% of the breeders used the milk obtained from water buffaloes for family needs, 36.1% sold the milk in the market, and 1.4% sold the milk to the merchants. While 75% of the breeders selling milk in the market sell both water buffalo and cow milk, 25% of the breeders sell only water buffalo milk in the market. Of the breeders, 7.4% stated that they evaluate the cream of milk separately. It was determined that the breeders processed the milk they obtained into yogurt (100%), cheese (98%), and butter (81.6%). Altınbaş (2003) has stated that 21.7% of the breeders process their water buffalo milk into yogurt, 3.3% into cheese, and 75% into both yogurt and cheese, and 76.5% have stated that they sell both of their products in the markets themselves.

### Care and Health Applications

Of the breeders, 43.5% prefer to give their water buffaloes a body bath and 89% prefer to give their water buffaloes a foot bath. Altınbaş (2003) has stated that the breeders gave their water buffaloes a body bath. In the same study, it has been reported that only 1.3% of the breeders gave their water buffaloes a footbath. This result differs from the data obtained in the present study and reveals that water buffalo breeders in Diyarbakır give importance to the footbath.

While 93% of the breeders participating in the survey stated that they disinfect their water buffalo barns, 7% of the breeders stated that they do not disinfect their barns. All the breeders who carry out disinfection use the calcination method as the disinfection method in the barns. Majority of the businesses (93.9%) combat internal and external parasites for their water buffaloes. It was determined that foot and mouth disease (98.6%) and lice (17.7%) are the most common diseases seen in farms. Çiftçi and Yılmaz (2020) have stated that 77.21% of the businesses encountered parasites and foot and mouth disease, 8.82% encountered parasitic diseases, brucella, and foot and mouth disease, and 13.97% encountered digestive system problems, parasites, foot, and mouth disease and strokes. Özger (2018) have reported that, in Iğdır, the most common diseases in businesses were 65% foot and mouth disease, 17.5% foot and mouth disease and smallpox, and 10% foot and mouth disease and jaundice.

Regarding the question, "From whom do you get information about diseases?", 18.4% of the breeders have responded that they benefit from their own experience, 44.9% from veterinarians, 0.7% from agricultural engineers, 34.7% from both their own experience and veterinarians and 1.4% from both veterinarians and agricultural engineers. Çiftçi and Yılmaz (2020) have reported that 11.76% of the breeders administer the drugs according to their own experience, 83.09% consult a veterinarian, and 5.15% of the breeders administer the drugs according to their own experience and consult a veterinarian.

### Data on Expectations and Problems of Farmers

While 79.6% of the water buffalo breeders who participated in the survey answered, 'Livelihood for the Family' as the reason for raising water buffalo, 42.2%

responded as 'Milk Quality', 5.4% as 'Hobby Purposes', 2% as 'Family Profession', 6.8%' as 'for Utilizing Subsidies', and 1.4% as 'For Meat'. Özdemir and Özdemir (2016) in their survey study in Bingöl province, have reported that 29% of the water buffalo breeders have stated they breed water buffaloes for meeting the daily needs of the household, 24% for family occupation, 13% for adapting to geographical conditions, 12% for their love for animals, 9% for earning their livelihood, 7%, for benefiting from subsidies, 5% for being their only source of income, 1% for having being trained in the profession, and %1 for having delicious products.

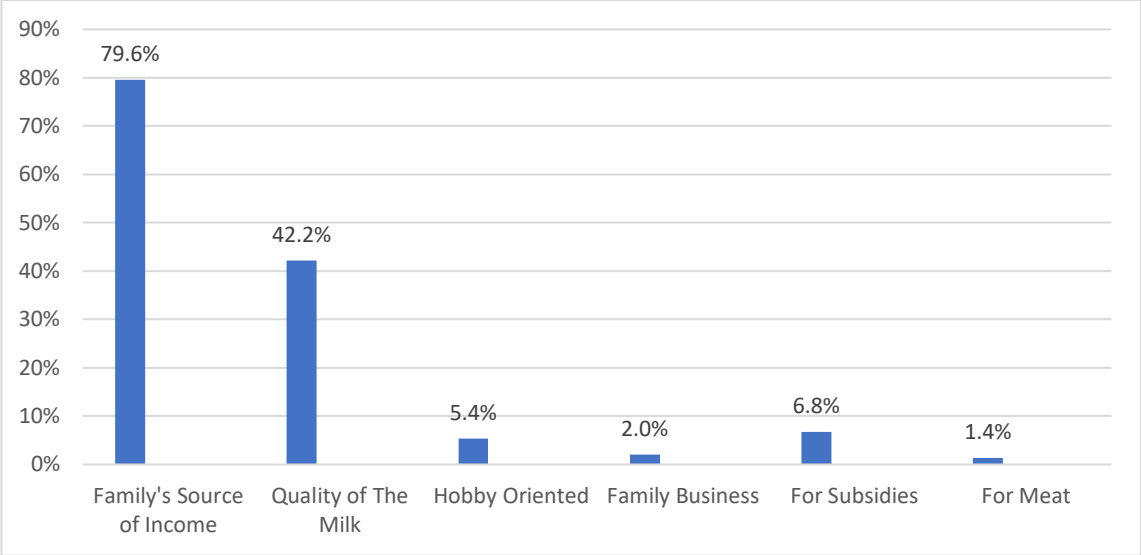


Figure 7. Reasons of breeders for breeding water buffalo (%)

While 51% of the breeders have stated that the current number of water buffaloes in their region has been decreasing and that will gradually decrease, 49% of the breeders think that the current number of water buffaloes in their region has been increasing and will increase over time. Regarding the question for the decrease in the number of water buffaloes in their region, 77.3% of the breeders have stated that the subsidies were not sufficient, 4% have stated that the water buffaloes were difficult to maintain, 8% have stated that they had low yields, 44% have stated that the pasture lands were not sufficient, 17.3% have stated that the feed costs were high, 2.7% have stated that the water buffaloes have a long gestational period and 2.7% of the breeders have stated that the Breeder Water Buffalo Breeders' Association does not accept membership. Regarding the reasons for the increase in the number of water buffaloes in their region, 81.9% of the breeders have stated that they had the opportunity to benefit from the existing subsidies, 6.9% have stated that the adaptation of the water buffalo to the region was easy, 13.9% have stated that the milk quality of the water buffalo was high, and 2.8% have stated that the water buffalo breeding contributed to the family's livelihood. Regarding the question, "In which subjects would you like to have more information?", 70% of the breeders have stated that they would like to have more information about benefiting from the existing subsidies, 15% have stated that they wanted to learn about animal health and 15% wanted to learn about farming practices. While 53.1% of the water buffalo breeders participating in the survey have been considering expanding their existing businesses, 46.9% have stated that they did not plan to expand their existing businesses. Regarding the reasons, of the breeders who plan to expand their businesses, 51.3% have stated that they wanted to benefit from the subsidies, 12.8% wanted to increase their income, and 7.7% wanted to produce more milk. When asked about the reasons of the breeders who do not plan to expand their existing business, 34.2% of the breeders found the barn capacity insufficient, 13.7% found the current operating capacity sufficient, 9.6% found the breeding costs high, 4.1% have mentioned low animal sales prices, 15.1% have stated that there was a shortage of caregivers, 20.5% have stated that the subsidies were not sufficient and 2.7% have stated that the water buffaloes had low yield.

## Conclusion

The low average number of water buffaloes per farm and the small barn capacities in the surveyed businesses cannot meet the high input prices in the businesses. Breeder organizations should assist their members in providing more affordable inputs. Increasing the operating capacity and modern water buffalo breeding should be encouraged, and resources should be provided to water buffalo breeders through banks or Agricultural Credit Cooperatives. The low productivity of the region's water buffalo breeds affects the milk and carcass production volumes. Breeding studies that will increase yield should be carried out effectively and breeders should be included in these breeding studies. To promote and market the products obtained from water buffalos, branding should be encouraged through organizations such as cooperatives, unions, etc. Promotional advertisements can be carried out in the media for the dissemination of breeding and the demand for water buffalo meat, milk, and products. Training should be provided to breeders on water buffalo care,

health, and breeding by union employees or engineers, and the shortcomings of traditional methods should be eliminated. The state should increase and maintain the existing subsidies for water buffalo breeding, provide inspections on subsidies and do the necessary work to inform the breeders about the developments related to the subsidies. Water buffalo breeding should be encouraged by presenting programs to encourage young entrepreneurs and engineers.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

This study is derived from Murat TURAN's master's degrees thesis. The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Morphological characterization of some Besni pepper (*Capsicum annuum* L.) genotypes in Kayseri conditions

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## Abstract

The pepper genetic resources, which is a widely produced and consumed vegetable in Turkey and the world, are faced with some threats arising from the environmental conditions and agricultural activities. Therefore, it is very important to protect pepper genetic resources and include them in breeding programs. During the production adventure of pepper in Turkey, pepper genotypes known by the name of the regions were developed in different regions such as Demre, Uşak, Karaisalı, and Arapkir pepper. One of them, Besni Pepper, is grown in and around Besni district of Adıyaman province and makes significant contributions to the regional economy. In this study, 26 pepper genotypes collected from the villages of Besni and Gölbaşı districts, and three control varieties were characterized according to 42 morphological traits. Pepper genotypes showed significant variation in terms of the characteristics considered. Principal Component Analysis (PCA) was applied to the investigated traits. The PCA analysis yielded 10 principal components explaining 86% of the total variation. The eigen values of 10 PC's varied from 10,50 to 1,10. The first three PC's explain 51,20% of the total variance. The variation between genotypes is mostly due to fruit characteristics such as fruit shape, fruit size, and blossom end shape. While the genotypes were divided into 4 groups in the cluster analysis, the pepper varieties used as control were separated from all genotypes and formed a separate group.

## Keywords

*Capsicum annuum,* Besni pepper, Morphological characterization, Variation

## Introduction

Genetic resources are the unique resource for the development of high-yielding varieties that are resistant to biotic and abiotic stress conditions to ensure the sustainability of plant production. Local populations that have adapted to their ecology for years and have survived and sustained against certain biotic and abiotic stress factors are important resources for plant breeding. Turkey has unique resources in terms of landraces of cultivated crops, which are formed as a consequence of selection by local producers and still show great diversity. Seeds or other multiplication materials are always the most important element in plant production. The yield or product quality of a plant is directly related to the seed genetic makeup. Although the cultural practices applied in plant production are maintained at the optimum level, yield and quality cannot go beyond the genetic limits determined by the seed genetic potential. Therefore, it is possible to achieve a significant increase in yield and quality by introducing new and superior plant varieties into agricultural production by using genetic diversity. Local genotypes are important genetic resources because they have unique gene pools and serve as important resources of genetic diversity for plant breeding and conserving biodiversity (Arslan, 2010; Balkaya et al., 2010).

Local populations are rich genetic resources in this respect and constitute starting materials that can be used to develop new cultivars. The use of these local genotypes/landraces as new varieties that satisfy consumer demands becomes possible by developing them in breeding programs. Local varieties that are not registered and selected (bred) by the local people are characterized by their special adaptation to the

environmental conditions in the cultivated area, and they are closely related to the lifestyles, knowledge and traditional uses of the societies that selected them and continued to cultivation (Negri, 2007).

Biological diversity has indispensable roles and importance in meeting the basic needs of people, especially food and clothing. It is estimated that 20% of global biodiversity is lost due to continuous and misuse of natural resources as well as pollution caused by human activities. Loss of biodiversity in any population reduces their ability to overcome biotic and abiotic stress conditions causing a reduction in yield and quality of crop plants. Turkey, which has important plant biodiversity, has been faced with the loss of biological diversity due to various adverse factors (natural and mankind activities). For this reason, the collection, preservation, characterization, and use of plant genetic resources are of crucial importance in sustainable agriculture and the environment (Davis et al., 1988; Özhatay et al., 2009).

Turkey, which has very different climate and soil characteristics due to its location, is located at the intersection of the Near East, the Mediterranean, and European gene centers, which are among the eight main plant genetic diversity centers. It is in the region where the Euro-Siberian, Mediterranean, and Iran-Turan plant geographical regions are located. Anatolia includes regions such as Mesopotamia (fertile-crescent) where agriculture was first practiced in the world. Therefore, Anatolia has become the diversity center and micro gene center of many cultivated plant species (Karagöz et al., 2020). According to Harlan, there are 5 micro-gene centers in Turkey where more than 100 species show wide variation (Demir, 1990). Moreover, a high degree of plant endemism has occurred, and 4.080 of the 12.476 plant species recorded in Turkey are endemic (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000; Vural, 2003; Erik and Tarıkahya, 2004; Özhatay and Kültür, 2006; Özhatay et al., 2009). However, these plant genetic resources are in danger of being lost by genetic erosion for some reasons. It is very important to protect the diversity for the sustainability of plant production, especially for the plant genetic resources of the cultivated species (Tan and İnal, 2003). Diversity in plant genetic resources is gradually decreasing due to reasons such as forest fire, erosion, increased land openings, replacing local varieties with bred modern varieties, urbanization, road construction, changes in agricultural systems and plant protection methods (intensive use of pesticides), and continuous supply of bulbous plants from nature. Countries that are aware of this danger faced in plant genetic resources have started studies on the collection, characterization, and preservation of resources (Tan, 1992). For example, in Bulgaria (Vesselinov et al., 1982), Peru (Gomez and Cuartero, 1984), Spain (Eshbaugh, 1988), Taiwan (Wang et al., 2000), Brazil (do Rêgo et al., 2011), Argentina (Occhiuto et al., 2014), Uganda (Nsabiyera et al., 2013) and Eritrea (Saleh et al., 2016), studies on collection and characterization of pepper germplasm have been carried out. Similarly, collection and characterization studies of local pepper genotypes in Turkey were carried out by Keleş (2007), Binbir and Baş (2010), Karağaç and Balkaya (2010), Bozokalfa and Eşiyok (2010), Baysal (2013), Çürük et al. (2015), Keleş et al. (2016), Başak (2019), Taş and Balkaya (2021) and Altuntaş (2021).

Pepper, an important member of the Solanaceae family, originated from America has a very wide distribution in the world (Vural et al., 2000). Pepper is a major vegetable species that was brought to Spain from the Americas with the travel of Columbus in the 1400s. In later times, it was reported that the pepper was increasingly distributed in the African and Asian continents by the Spanish and Portuguese merchants through trade and exploration routes. Pepper is extensively consumed as a spice or fresh vegetable in many parts of the world. Besides giving taste and color to the food, the fruit of the pepper is an important source of vitamins and minerals for humans. However, pepper juice and extracts are used in the cosmetic industry and pharmacology (Pernezny et al., 2003). Peppers have been classified according to different approaches by researchers. For the taxonomic classification in this genus, several alternative approaches such as geographical, ethnobotanical crossability, numerical taxonomy, cytogenetics, and biochemical data have been used (Pickersgill, 1991; Nicolai et al., 2013). The number of species belonging to the Capsicum genus, which was 38, has been updated to 43 with the addition of 5 new species identified by taxonomists (Barboza et al., 2019). Five of them were domesticated through prominent events at different locations in America (Heiser and Smith, 1953; Nicolai et al., 2013; Olmstead et al., 2008). According to), These are *Capsicum annum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L, and *C. pubescens* R&P (Samos and Kundt, 1984). *C. annuum* is the most common and economically important species of Capsicum in the world. It is a diploid and self-fertile crop with 24 chromosomes (Gyulai et al., 2000). However, two classifications are generally accepted as *C. annum* and *C. frutescens* groups. Plants belonging to the *C. annuum* species are known as monoecious and produce a single flower from each branching point. *C. frutescens*, on the other hand, is known as a perennial and forms more than one flower in bunches (Purseglove, 1974). Although Turkey's pepper production varies every year, it maintains its place in the top three along with China and Mexico (Fao, 2020). Peppers are grown in both protected (tunnels and greenhouse) cultivation and open field conditions in Turkey. According to the data of 2020 in Turkey; 2.625.669 tons of fresh pepper (long pointed, capia, bell, charleston) and 26.000 tons of dry pepper were produced (Tuik, 2020).

As stated above, plant germplasm may undergo genetic erosion due to different reasons, and the collection, characterization, and evaluation of them are important issues in the sustainability of plant production. In the previous researches, some of which were cited above, in Turkey, studies were carried out on pepper genotypes collected from different regions of Turkey. However, it has been determined that no studies have been carried out on the local pepper genotype known as Besni Pepper. Therefore, 26 Besni Pepper genotypes cultivated for many years in a restricted area in Turkey, were collected and morphologically characterized for 42 traits.

## Materials and Methods

### Plant materials

In this study, 29 pepper genotypes were used as plant material. 20 of them were collected from different villages of Besni district and 6 of them were collected from Gölbaşı district in 2019. Yalova Corbaci, Sera Demre and Cırgalan peppers were used as controls (Table 1).

Table 1. Pepper genotypes used in the experiment and their source

| Genotype | Source | Genotype | Source |
|---|---|---|---|
| B1 | Oyratlı Village/Besni | B16 | Oyratlı Village /Besni |
| B2 | Oyratlı Village /Besni | B17 | Oyratlı Village /Besni |
| B3 | Oyratlı Village /Besni | B18 | Oyratlı Village /Besni |
| B4 | Oyratlı Village /Besni | B19 | Oyratlı Village /Besni |
| B5 | Oyratlı Village /Besni | B20 | Toklu Village /Besni |
| B6 | Oyratlı Village /Besni | G1 | Gölbaşı |
| B7 | Oyratlı Village /Besni | G2 | Gölbaşı |
| B8 | Oyratlı Village /Besni | G3 | Gölbaşı |
| B9 | Oyratlı Village /Besni | G4 | Maltepe Village /Gölbaşı |
| B10 | Besni | G5 | Maltepe Village /Gölbaşı |
| B11 | Oyratlı Village /Besni | G6 | Maltepe Village /Gölbaşı |
| B12 | Oyratlı Village /Besni | C1 (Cırgalan) | Erciyes University |
| B13 | Çamurcu Village /Besni | C2 (Yalova Çorbacı) | Erciyes University |
| B14 | Çamurcu Village /Besni | C3 (Sera Demre) | Erciyes University |
| B15 | Oyratlı Village /Besni | | |

### Plant production and cultural practices

This study was carried out at the Erciyes University Agricultural Faculty in 2021**.** Twenty-five seeds from each pepper genotype were sown in multi-pots filled with a 2:1 peat and perlite mixture (2v:1v) on 02.04.2021 in an unheated greenhouse. Three seedlings with 3-4 true leaves from each genotype were transplanted in soil in an unheated greenhouse on May 5, 2021. The spacing was 100 cm (between rows) x 50 cm (within the row). Before transplanting, a drip irrigation system was established, and the soil surface was covered with black plastic mulch. Fertilizer was applied by fertigation method as 12 kg N/da, 5 kg P/da, 15 kg K/da, 5 kg Ca/ and 3 kg Mg/da (Şalk et al., 2008).

### Morphological characterization

Pepper genotypes were morphologically characterized for 42 traits according to the descriptor list of pepper published by UPOV (International Union for Conservation of New Plant Varieties) and modified by Keleş (2007) (Table 2).

Stem diameter (mm), fruit length (cm), fruit diameter (cm), fruit pedicel length (cm), fruit wall thickness (mm) were measured with a digital caliper.

Cotyledon width, cotyledon length, leaf blade length (mm), leaf width, and the length of the petiole (mm) were measured with a ruler. Yield and fruit weight (g) was measured with a scales

Other parameters were evaluated visually.

### Statistical analysis

Observed traits were presented as numbers corresponding to the phenotype presented in the descriptor list. Measured characteristics in plants were presented as a mean of 3 measurements while measured fruit and flower characteristics were presented as a mean of 10 measurements. SPSS program was used in the analysis of the data. The data were first subjected to Principal Component Analysis (PCA) and principal component (PC) axes of genotypes were obtained (Sneath and Sokal, 1973). PC axes, variation and cumulative variation ratios, and factor coefficients were determined. The data was subjected to cluster analysis to determine the relationship between genotypes using SPSS software using Between-group linkage.

## Results

In this study, 26 pepper genotypes collected from Adıyaman province and 3 control pepper varieties were morphologically characterized for 26 observed and 16 measured characteristics. There was no variation between pepper genotypes in stem background color (all green), leaf shape (all lanceolate), flower position (all pendant), calyx margin (all dentate), coloration on the calyx (all absent), petal shape (all campanulate) and color (all white).

The cotyledon length ranged from 25,14 mm to 34,01 mm, and the mean cotyledon length was calculated as 28,29 mm. The longest cotyledon length was recorded in G3 (34,01 mm) and the shortest cotyledon was recorded in C3 with 25,14 mm. Pepper genotypes varied in cotyledon width. Pepper genotype B7 had the narrowest cotyledon with 7,62 mm while G3 had the widest cotyledon with 11,18 mm and the cotyledon width mean was calculated as 8,89 mm (Table 3).

Anthocyanin formation in hypocotyl showing variation was scored between 1-9. The highest anthocyanin formation was observed in B19 with 8, while the lowest anthocyanin formation on hypocotyl was determined in genotypes C1 and C3. Genotypes also differed in terms of anthocyanin formation on stems and nodes. While the lowest anthocyanin formation on the stem was observed in genotypes C1 and C3, the most intense anthocyanin formation was observed in genotype B19. While the highest anthocyanin concentration in the nodes was observed in B19 (9), the lowest anthocyanin intensity was observed in B6, B7, B8, B18, G3, G4, G5, and C3 (1) (Table 3).

Plants were segregated into two groups as compact/intermediate (11) and erect (18) according to the plant growth habits. While all the control genotypes were in the erect growing group, 11 of the collected

pepper genotypes were in the compact growing group. Plant height varying from 103,33 cm to 73,33 cm showed significant variation between genotypes. Control plants produced taller plants than the collected pepper genotypes. While the average plant height of the control plants was 100,90 cm, the average plant height of the other genotypes was calculated as 88,51 cm. The tallest plants were recorded in C3 (103,33 cm) and the shortest plant was determined in B11 (73,33 cm).

Table 2. Descriptor list for pepper

| Traits | Explanation |
|---|---|
| Anthocyanin on hypocotyl | Absent (1); present (9) |
| Anthocyanin on stem | Absent (1); present (9) |
| Cotyledon shape | Triangle (1); ovate (2); spear shape (3); ong triangle (4) |
| Cotyledon width and length | (cm) |
| Plant growth habit | Prostrate (3); intermediate (compact) (5); erect (7);oOther (9 specify) |
| Leaf color | Yellow (1); light green (2); green (3); dark green (4)<br>Light purple (5); purple (6); variegated (7); other (8) |
| Leaf shape | Triangle (1); ovate (2); lanceolate (3) |
| Leaf margin | Absent or very light (1); intermediate (2); strong (3) |
| Leaf pubescence | Sparse (1); intermediate (2); dens (3) |
| Leaf length and width | (cm) |
| Petiole length | (cm) |
| Stem background color | Green (1); green with purple stripes (2); purple (3); other (4 |
| Nodal anthocyanin | Absent (1); Present (9) |
| Intensity of nodal anthocyanin | Very little (1); less (3); intermediate (5); strong (7); very strong (9) |
| Stem shape | Cylindrical (1), angled (2); flattened (3) |
| Stem pubescence | Sparse (3), intermediate (5) dense (7) |
| Stem diameter | (mm) |
| Flower position | Pendant (3); intermediate (5) erect (7) |
| Calyx margin | Entire (1); intermediate (2); dentate (3); other (4) |
| Calyx annular constriction | Absent (0); present (1) |
| Calyx pigmentation | Absent (0); present (1) |
| Corolla color | White (1); light yellow (2); yellow (3); yellow-green (4); purple with white base (5); white with purple base (6) white with purple margin (7); purple (8); other (9) |
| Corolla shape | Rotate (1); campanulate (2); other (3) |
| Anther color | White (1); yellow (2); pale blue (3); blue (4); purple (5); other (6) |
| Fruit shape | Elongate (1); almost round (2); triangular<br>(3); campanulate (4); blocky (5); other (6) |
| Fruit shape at pedicel attachment | Acute (1); obtuse (2); truncate (3); cordate (4); lobate (5) |
| Neck at base of fruit | Absent (0); present (1) |
| Fruit blossom end shape | Pointed (1); blunt (2); sunken (3); sunken and pointed (4), other (5) |
| Fruit blossom end appendage | Absent (0); present (1) |
| Placenta size | Small (3); intermediate (5); large (7) |
| Fruit cross-sectional corrugation | Slightly corrugated (3); intermediate (5); corrugated (7) |
| Fruit length and diameter | (cm) |
| Single fruit weight | Average of 10 mature fruits (g) |
| Fruit wall thickness | (mm) |
| Fruit color at intermediate stage | White (1); yellow (2); green (3); orange (4); purple (5); deep purple (6); other (7) |
| Number of loculus | (Number) |
| Fruit surface | Smooth (1); semi-wrinkled (2); wrinkled (3) |
| Plant height | (cm) |
| Fruit pedicel length | (cm) |
| Fruit shape (longitudinal section) | Round (1); heart shaped (2); square (3); rectangle (4); trapezoidal (5); triangle (6); narrow triangle (7);horn-shaped (8) |
| Yield | (g/plant) |
| Fruit number | (number) |
| Ripe fruit pungency | Sweet (1), pungent (2) |

Table 3. Observed and measured seedling and plant characteristics

| Genotypes | CL (mm) | CW (mm) | AH | AS | NAF | PGH | SD (mm) | PL (cm) | SS | SP |
|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 30,84 | 9,19 | 6 | 6 | 5 | 5 | 18,6 | 86,7 | 1 | 5 |
| B2 | 28,06 | 8,73 | 6 | 6 | 5 | 5 | 20,1 | 94,3 | 1 | 7 |
| B3 | 28,83 | 8,58 | 5 | 4 | 5 | 5 | 22,5 | 81,7 | 1 | 7 |
| B4 | 26,41 | 8,13 | 4 | 5 | 5 | 5 | 17,9 | 85,0 | 1 | 7 |
| B5 | 25,64 | 7,64 | 5 | 4 | 3 | 7 | 19,8 | 90,0 | 1 | 7 |
| B6 | 28,69 | 8,74 | 5 | 2 | 1 | 5 | 19,7 | 94,0 | 1 | 5 |
| B7 | 25,4 | 7,62 | 4 | 2 | 1 | 5 | 23,2 | 101,7 | 2 | 5 |
| B8 | 29,29 | 9,07 | 4 | 2 | 1 | 5 | 23,0 | 80,0 | 3 | 3 |
| B9 | 25,95 | 8,68 | 6 | 5 | 5 | 5 | 22,4 | 88,3 | 3 | 5 |
| B10 | 28,12 | 8,61 | 5 | 5 | 5 | 5 | 18,0 | 75,0 | 3 | 7 |
| B11 | 29,73 | 8,92 | 4 | 6 | 5 | 5 | 18,4 | 73,3 | 1 | 3 |
| B12 | 28,61 | 8,28 | 6 | 6 | 5 | 7 | 18,8 | 75,0 | 3 | 3 |
| B13 | 29,69 | 9,16 | 4 | 5 | 3 | 7 | 20,0 | 76,0 | 1 | 7 |
| B14 | 26,77 | 8,62 | 3 | 4 | 3 | 7 | 23,0 | 85,0 | 1 | 7 |
| B15 | 28,21 | 8,62 | 6 | 4 | 3 | 7 | 16,0 | 85,0 | 1 | 3 |
| B16 | 28,45 | 8,55 | 4 | 4 | 3 | 7 | 20,7 | 93,0 | 1 | 3 |
| B17 | 29,31 | 8,44 | 5 | 5 | 5 | 7 | 18,1 | 83,3 | 1 | 3 |
| B18 | 26,93 | 8,69 | 3 | 3 | 1 | 7 | 21,9 | 98,3 | 3 | 5 |
| B19 | 28,23 | 9,09 | 8 | 8 | 7 | 7 | 21,6 | 86,7 | 3 | 5 |
| B20 | 31,05 | 10,27 | 4 | 4 | 5 | 7 | 18,0 | 100,0 | 1 | 3 |
| G1 | 28,45 | 9,11 | 4 | 4 | 3 | 7 | 21,9 | 91,7 | 1 | 3 |
| G2 | 25,83 | 9,00 | 5 | 5 | 5 | 7 | 18,7 | 86,7 | 2 | 3 |
| G3 | 34,01 | 11,18 | 4 | 0 | 1 | 7 | 20,5 | 90,0 | 1 | 3 |
| G4 | 32,06 | 10,64 | 6 | 2 | 1 | 7 | 20,8 | 80,0 | 3 | 5 |
| G5 | 26,65 | 9,25 | 4 | 4 | 1 | 7 | 21,5 | 96,7 | 1 | 3 |
| G6 | 27,00 | 8,43 | 7 | 4 | 3 | 5 | 21,4 | 86,7 | 1 | 3 |
| C1 | 32,97 | 9,67 | 1 | 1 | 3 | 7 | 14,3 | 97,7 | 1 | 3 |
| C2 | 29,08 | 8,85 | 5 | 5 | 5 | 7 | 10,9 | 101,7 | 1 | 3 |
| C3 | 25,14 | 8,14 | 1 | 1 | 1 | 7 | 16,0 | 103,3 | 1 | 3 |
| Mean | 28,29 | 8,89 | | | | | 19,59 | 88,51 | | |
| Minimum | 25,14 | 7,62 | | | | | 10,93 | 73,33 | | |
| Maximum | 34,01 | 11,18 | | | | | 23,23 | 103,33 | | |

CL: Cotyledon length; CW. Cotyledon width, AH: Anthocyanin on hypocotyl; AFS: Anthocyanin on stem; NAF: Nodal anthocyanin formation; SD: Stem diameter; PGH: Plant growth habit; PL: Plant length; SS: Stem shape; SP: Stem pubescence.

A significant variation was detected in stem diameter. While the pepper genotype C2 had the lowest stem diameter of 10,93 mm, the largest stem diameter was measured in the B7 genotype with 23,23 mm, and the mean stem diameter was calculated as 19,59 mm. In terms of stem shape, genotypes were divided into three groups as 20 cylindrical, 2 angular, and 7 flattened. All three control pepper cultivars had flattened stems, while the other genotypes had all three stem shapes. As in stem shape, genotypes formed three groups in terms of stem hairiness as sparse (15), intermediate (7), and dense (7). While sparse pubescence was observed in three control cultivars, collected pepper genotypes showed sparse (12), intermediate (7), and dense (7) pubescence on the stem (Table 3).

The measurement and observation in leaves and anther color are given in Table 4. The petiole length varied between 4,16 cm and 9,83 cm and the average petiole length was calculated as 7,33 cm. The longest and shortest petiole were measured in B14 and G2, respectively. Leaf color was recorded as dark green (4) in B20, G2, G3, G4, G5, G6, and green for all other genotypes. G3 and G4 genotypes had ovate leaves, while other genotypes had lanceolate leaves. Genotypes are divided into two groups according to lamina margin as entire and undulate. B4, B7, B12, B13, B15, C3 had entire lamina margins while other genotypes had undulate lamina margins.

In leaf pubescence, sparse pubescence was observed in 24 genotypes, medium pubescence in three genotypes, and dense pubescence in one genotype (G1). A difference in leaf length was 7,33 cm between genotypes with the longest and the shortest leaves. The longest and shortest leaves were measured as 11,66 and 19,00 cm in genotypes G2 and G4 taken from Gölbaşı district, respectively, and the mean leaf length was calculated as 15,1 cm. The variation in leaf width was lower than that in leaf length. The leaf width varied between 6,00 cm (B7) and 10,83 cm (G4) and the average leaf width was calculated as 8.4 cm. Genotypes were divided into three groups according to anther color: blue (1), pale blue (23), and purple (5). B7, B18, G3, G4, C3 had purple anthers, B20 had blue anthers and the other 23 genotypes had pale blue anthers (Table 4).

The yield and some measured fruit characteristics are presented in Table 5. Yield per plant ranged from 155,33 g/plant to 795,83 g/plant. The difference between the lowest and the highest yielding genotype

was approximately five folds. All three control cultivars had lower yields than the other genotypes. While the highest yield was recorded in the genotype G5 with 795,83 g/plant, the lowest yield was obtained from C2 with 155,33 g/plant.

Table 4. Leaf characteristics and anther color

| Genotypes | PL (cm) | LC | LS | LMS | LP | LL (cm) | LW (cm) | AC |
|---|---|---|---|---|---|---|---|---|
| B1 | 7,33 | 3 | 3 | 2 | 1 | 15,33 | 8,33 | 3 |
| B2 | 7,00 | 3 | 3 | 2 | 1 | 14,00 | 8,33 | 3 |
| B3 | 7,33 | 3 | 3 | 2 | 1 | 12,66 | 7,33 | 3 |
| B4 | 7,00 | 3 | 3 | 1 | 1 | 14,00 | 8,00 | 3 |
| B5 | 7,33 | 3 | 3 | 2 | 2 | 13,66 | 7,83 | 3 |
| B6 | 7,83 | 3 | 3 | 2 | 1 | 15,66 | 9,00 | 3 |
| B7 | 6,83 | 3 | 3 | 1 | 1 | 12,66 | 6,00 | 5 |
| B8 | 8,83 | 3 | 3 | 2 | 1 | 15,33 | 8,00 | 3 |
| B9 | 8,00 | 3 | 3 | 2 | 1 | 16,83 | 9,00 | 3 |
| B10 | 6,50 | 3 | 3 | 2 | 1 | 15,66 | 8,66 | 3 |
| B11 | 8,16 | 3 | 3 | 2 | 1 | 15,50 | 8,50 | 3 |
| B12 | 7,50 | 3 | 3 | 1 | 1 | 14,83 | 7,83 | 3 |
| B13 | 9,66 | 3 | 3 | 1 | 1 | 17,66 | 8,83 | 3 |
| B14 | 9,83 | 3 | 3 | 2 | 1 | 17,33 | 9,33 | 3 |
| B15 | 6,33 | 3 | 3 | 1 | 2 | 15,33 | 7,83 | 3 |
| B16 | 6,83 | 3 | 3 | 2 | 1 | 16,33 | 9,33 | 3 |
| B17 | 7,33 | 3 | 3 | 2 | 2 | 14,50 | 7,83 | 3 |
| B18 | 9,33 | 3 | 3 | 2 | 2 | 15,33 | 9,50 | 5 |
| B19 | 7,83 | 3 | 3 | 2 | 1 | 19,00 | 10,00 | 3 |
| B20 | 6,66 | 4 | 3 | 2 | 1 | 15,33 | 8,33 | 4 |
| G1 | 7,50 | 3 | 3 | 2 | 3 | 14,66 | 8,33 | 3 |
| G2 | 4,16 | 4 | 3 | 2 | 1 | 11,66 | 7,33 | 3 |
| G3 | 8,33 | 4 | 2 | 2 | 1 | 14,50 | 8,33 | 5 |
| G4 | 8,33 | 4 | 2 | 2 | 1 | 19,00 | 10,83 | 3 |
| G5 | 9,00 | 4 | 3 | 2 | 1 | 14,00 | 8,66 | 3 |
| G6 | 8,33 | 4 | 3 | 3 | 1 | 14,66 | 8,00 | 3 |
| C1 | 4,50 | 3 | 3 | 2 | 1 | 15,33 | 8,33 | 3 |
| C2 | 4,16 | 3 | 3 | 2 | 1 | 15,33 | 8,33 | 3 |
| C3 | 5,00 | 3 | 3 | 1 | 1 | 12,00 | 7,00 | 5 |
| Mean | 7,33 | | | | | 15,21 | 8,42 | |
| Minimum | 4,16 | | | | | 11,66 | 6,00 | |
| Maximum | 9,83 | | | | | 19,00 | 10,83 | |

PL: Petiole length; LC: Leaf color; LS: Leaf shape; LMS: Leaf margin shape; Leaf pubescence; LL Leaf Length; Leaf width

The average yield of control cultivars and other pepper genotypes was calculated as 213,40 g/plant and 448,37 g/plant, respectively. Variation in single fruit weight was also significant. The heaviest fruit weight was 153,44 g in genotypes B20, while the lightest fruit weight was 4,66 g in B7 and the average single fruit weight was calculated as 58,77 g. The number of fruits per plant varied between 26,22 and 4,33 fruit/plant. The highest and lowest fruit number were recorded in B7 and B14 with 26,22 and 4,33 fruit/plant, respectively.

As in yield parameters, fruit length and fruit diameter also showed significant variation. The variation in fruit diameter (about 9 folds) was higher than the variation in fruit length (3 folds). The longest and shortest fruits were determined in genotypes B8-B9 and C3 with 4,67 and 15,00 cm, respectively. While C1 had fruit length close to genotypes taken from Adıyaman, C2 and C3 had longer fruits. Fruit diameter ranged from 0,97 cm to 5,40 cm and the average fruit diameter was calculated as 3,92 cm. The largest and lowest fruit diameters were measured as 0,97 cm and 5,40 cm, respectively, in B7 and G2 genotypes. The fruit diameter of the genotypes taken from Adıyaman was two times higher than the fruit diameter of the control cultivars (Table 5).

The fruit wall thickness ranged from 0,97 mm to 2,93 mm, and the average fruit wall thickness was 1,93 mm. The thinnest fruit wall was determined in the B7 genotype (0,97 mm), and the thickest fruit wall was determined in the B20 genotype (2,93 mm). The minimum and maximum fruit pedicel length of pepper genotypes were measured as 3,33 and 6,50 cm, respectively, and the mean fruit pedicel length was calculated as 4,33 cm (Table 5).

The results of the observations on fruit characteristics are given in Table 6. In terms of placenta size, it was observed that 13 of the genotypes had large, 11 intermediates, and 5 small placentas. Control cultivars had smaller placentas than other pepper genotypes. Three different fruit shapes (elongated; campanulate and blocky) were observed in the evaluated pepper genotypes. With 19 genotypes, campanulate fruit shape was found as a dominant fruit shape, 7 genotypes had elongated fruits and 3 genotypes had blocky fruits. Pepper genotypes are divided into two groups as smooth (10) and semi-wrinkled (19) in terms of fruit surface structure. In terms of fruit color in the intermediate

stage, while the majority of pepper genotypes were green, 3 of them had yellow fruit color. The number of locules in pepper genotypes ranged from one to four. In pepper genotypes collected from Adıyaman, genotypes had 4 locules except for B7 and B2 (2). While neck formation was not observed in 7 of the pepper genotypes, neck formation was detected in 22 of them. Pepper genotypes were divided into four groups as pointed, blunt, sunken, and pointed according to the blossom end shape. The most commonly observed blossom end shape was sunken in 21 genotypes. Four different pedicel attachment patterns were observed in pepper genotypes. These are lobate (17), obsute (6), cordate (5), and acute (1). Fruit blossom appendage was observed in 26 of the pepper genotypes, but not in three of them. According to the pungency in ripe fruit determined by tasting, 26 of the pepper genotypes were found to be pungent and 3 of them were sweet. Genotypes were divided into three groups in terms of fruit cross-section shape as slightly corrugated (8), medium (19), and corrugated (2).

Principal Component Analysis (PCA) was applied to the investigated traits and the principal components, eigen values, variance, and total variance are presented in Table 7. In PCA, factors with an eigen value greater than 1 were considered significant (Dunteman, 1989; Karaağaç and Balkaya, 2010). As a result of principal component analysis, 10 PC related to 37 morphological features were obtained. These PC's represent 86.38% of the total variance. The eigen values of 10 PC's varied from 10,50 to 1,10. The first three PC's explain 51,20% of the total variance. The first PC axis accounted for 28,38% of the variation, whereas the second and third axes accounted for 12,32% and 10,50%, respectively (Table 7). In the PCA, it has been reported that the first three axes should explain more than 25% of the variation (Mohammadi and Prasanna, 2003).

Table 5. Yield and fruit characteristics based on measurement

| Genotypes | TFW (g/plant) | SFW (g) | FN (number) | FL (cm) | FD (cm) | FWT (mm) | FPL (cm) |
|---|---|---|---|---|---|---|---|
| B1 | 439,00 | 72,83 | 7,44 | 7,33 | 4,52 | 1,83 | 4,17 |
| B2 | 470,09 | 56,72 | 7,22 | 6,33 | 4,02 | 1,80 | 4,17 |
| B3 | 451,70 | 69,91 | 7,11 | 7,83 | 5,19 | 2,33 | 4,17 |
| B4 | 369,09 | 61,36 | 5,67 | 8,17 | 4,73 | 2,40 | 3,67 |
| B5 | 406,19 | 51,02 | 6,33 | 6,00 | 4,36 | 1,73 | 4,33 |
| B6 | 577,40 | 55,14 | 7,67 | 7,33 | 4,52 | 2,17 | 4,83 |
| B7 | 479,70 | 4,66 | 26,22 | 6,33 | 0,97 | 0,97 | 5,67 |
| B8 | 481,69 | 71,26 | 5,78 | 4,83 | 4,04 | 2,10 | 3,83 |
| B9 | 553,00 | 62,58 | 6,00 | 4,83 | 3,66 | 1,70 | 4,33 |
| B10 | 382,20 | 68,07 | 7,44 | 6,17 | 4,19 | 1,67 | 4,00 |
| B11 | 441,09 | 57,07 | 8,33 | 5,00 | 4,38 | 1,70 | 4,00 |
| B12 | 530,33 | 48,40 | 7,44 | 7,00 | 4,44 | 2,03 | 3,33 |
| B13 | 418,00 | 64,62 | 5,00 | 5,50 | 4,26 | 1,87 | 3,50 |
| B14 | 360,00 | 61,77 | 4,33 | 6,33 | 4,43 | 1,70 | 4,50 |
| B15 | 420,90 | 60,14 | 6,67 | 6,83 | 5,27 | 2,27 | 4,17 |
| B16 | 427,83 | 54,67 | 7,00 | 4,67 | 2,78 | 1,40 | 3,83 |
| B17 | 516,09 | 59,72 | 6,44 | 8,50 | 5,08 | 2,33 | 3,83 |
| B18 | 470,60 | 59,40 | 6,44 | 5,00 | 3,53 | 1,33 | 4,00 |
| B19 | 414,90 | 68,28 | 6,00 | 7,83 | 5,06 | 2,10 | 3,33 |
| B20 | 608,00 | 153,44 | 4,89 | 9,50 | 4,95 | 2,93 | 6,50 |
| G1 | 463,09 | 68,67 | 8,00 | 8,33 | 4,66 | 2,40 | 3,67 |
| G2 | 460,83 | 85,17 | 4,89 | 6,83 | 5,40 | 2,87 | 3,67 |
| G3 | 624,33 | 56,50 | 12,56 | 8,17 | 4,19 | 1,97 | 5,00 |
| G4 | 427,60 | 61,21 | 4,67 | 6,67 | 4,13 | 1,77 | 6,00 |
| G5 | 795,83 | 27,44 | 17,22 | 8,33 | 3,04 | 2,40 | 3,67 |
| G6 | 371,50 | 67,95 | 5,89 | 6,50 | 3,92 | 1,77 | 4,67 |
| C1 | 238,33 | 24,77 | 10,00 | 6,17 | 2,60 | 1,53 | 4,50 |
| C2 | 155,33 | 20,64 | 5,67 | 13,83 | 1,10 | 1,20 | 6,17 |
| C3 | 248,09 | 31,10 | 6,78 | 15,00 | 1,40 | 1,60 | 5,33 |
| Mean | 448,37 | 58,77 | 7,76 | 7,28 | 3,92 | 1,93 | 4,37 |
| Minimum | 155,33 | 4,66 | 4,33 | 4,67 | 0,97 | 0,97 | 3,33 |
| Maximum | 795,83 | 153,44 | 26,22 | 15,00 | 5,40 | 2,93 | 6,50 |

TFW: Total fruit weight; SFW: Single fruit weight; FL: Fruit length; FD: Fruit diameter; FN: Fruit number; FWT: Fruit wall thickness; FPL: Fruit pedicel length.

Table 6. Observational fruit characteristics

| Genotypes | PS | FS | FSr | FCGM | LN | NFP | BES | PAS | FBEA | FCSS | RFP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 5 | 3 | 2 | 3 | 4 | 1 | 4 | 5 | 1 | 5 | 2 |
| B2 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B3 | 7 | 3 | 1 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B4 | 5 | 3 | 1 | 3 | 4 | 1 | 3 | 4 | 1 | 3 | 2 |
| B5 | 5 | 4 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B6 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B7 | 3 | 1 | 1 | 3 | 1 | 0 | 1 | 2 | 1 | 3 | 2 |
| B8 | 7 | 3 | 1 | 3 | 4 | 1 | 3 | 4 | 1 | 3 | 2 |
| B9 | 5 | 3 | 1 | 3 | 4 | 0 | 3 | 4 | 1 | 3 | 2 |
| B10 | 7 | 3 | 1 | 3 | 4 | 1 | 3 | 5 | 1 | 3 | 2 |
| B11 | 5 | 4 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 7 | 2 |
| B12 | 5 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B13 | 5 | 4 | 2 | 3 | 4 | 1 | 4 | 5 | 1 | 5 | 2 |
| B14 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B15 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B16 | 5 | 3 | 1 | 3 | 4 | 0 | 3 | 2 | 1 | 3 | 2 |
| B17 | 5 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B18 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 4 | 1 | 5 | 2 |
| B19 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| B20 | 7 | 1 | 1 | 3 | 2 | 1 | 1 | 2 | 0 | 3 | 2 |
| G1 | 5 | 1 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| G2 | 7 | 3 | 1 | 2 | 4 | 1 | 4 | 5 | 0 | 5 | 2 |
| G3 | 5 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 5 | 2 |
| G4 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 2 | 1 | 5 | 2 |
| G5 | 3 | 1 | 1 | 2 | 4 | 0 | 2 | 2 | 1 | 3 | 2 |
| G6 | 7 | 3 | 2 | 3 | 4 | 1 | 3 | 5 | 1 | 7 | 2 |
| C1 | 3 | 1 | 2 | 3 | 3 | 0 | 3 | 4 | 1 | 3 | 1 |
| C2 | 3 | 1 | 2 | 2 | 1 | 0 | 1 | 1 | 0 | 3 | 1 |
| C3 | 3 | 1 | 2 | 3 | 1 | 0 | 1 | 2 | 1 | 3 | 1 |

PS: Placenta size; FS: Fruit shape; FSr: Fruit surface; FCGM: Fruit color at green maturity; LN: Loculus number, NFP: Neck formation on pedicel; BES: Blossom end shape; PAS: Pedicel attachment shape FBEA: Fruit blossom end appendage; FCSS: Fruit cross-section shape; RFP: Ripe fruit pungency

Table 7. The number of factors related to eigen value statistics determined by principal component analysis and percentages of variance explained

| | Components | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
| Eigen values | 10,50 | 4,56 | 3,88 | 3,26 | 2,48 | 1,81 | 1,64 | 1,51 | 1,22 | 1,10 |
| % of variance | 28,38 | 12,32 | 10,50 | 8,80 | 6,71 | 4,89 | 4,43 | 4,07 | 3,30 | 2,97 |
| Cumulative % | 28,38 | 40,70 | 51,20 | 60,00 | 66,72 | 71,61 | 76,04 | 80,11 | 83,41 | 86,38 |

Correlation coefficients of 10 PCs explaining 86% of the total variation are given in Table 8. According to the results of the current study, it has been determined that the traits in the first three PC's that explain a significant portion of the total variation and that have a coefficient value above 0,3 should be considered (Brown, 1991). In the first PC, which constitutes 28% of the total variation, the contribution of 11 characteristics to the variation was positively high, while the contribution of 5 traits was negatively significant. Of the 16 features that contributed significantly to the variation in the first PC, 6 were measured and 10 were observed characteristics. In PC1, locus number, fruit diameter, fruit shape, blossom end shape, fruit neck formation, fruit pedicel attachment shape, pungency, placenta size, stem pubescence, fruit cross-section shape, anthocyanin formation on the hypocotyl contributed positively to the variation, while the contribution of plant height, fruit height, fruit stem length, anther color and fruit number per plant to variation was negatively significant. The traits with high coefficients in the second principal component were cotyledon length (0,887) leaf color (0,813), cotyledon width (0,622), fruit weight, and leaf shape (0,646). In the third main component, while the blossom end appendage, petiole length, stem diameter, and fruit color at green maturity contributed positively to the variation, presence of nodal anthocyanin, nodal anthocyanin density, and fruit wall thickness contributed significantly negative (Table 8).

To better understand the overall diversity among pepper genotypes, the data were subjected to Cluster analysis revealing genetic similarities, and groups are presented Figure 1. Pepper genotypes did not group

according to their origin. Pepper genotypes were primarily divided into two groups as the first main group containing three control varieties (Çırgalan, Demresivri and Yalova corbacı), and the second main group containing 26 other genotypes collected from different villages of Besni and Gölbaşı districts. The second main group divided into two subgroups as the first subgroup contains B6, B9, G3, G5, and G20 and the second subgroup contains the other 21 genotypes. The 21 pepper genotypes in the second subgroup were divided into two groups, which included 9 (2-2-1) and 12 (2-2-2) pepper genotypes. In the clustering analysis, the most distant genotypes were C2 and B13 among all genotypes, while the two most distant genotypes among the collected genotypes were B13 taken from Çamurcu village and G5 from Maltepe village (Figure 1).

Table 8. Correlation coefficients between investigated characteristics and factors

| | Correlation coefficient | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Traits | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
| Number of loculus | **0,895** | | 0,104 | | | -0,335 | | | -0,101 | -0,123 |
| Fruit diameter | **0,848** | 0,257 | -0,284 | -0,114 | 0,237 | | | -0,159 | | |
| Fruit shape | **0,813** | -0,162 | | 0,146 | | | -0,236 | | 0,132 | -0,224 |
| Blossom end shape | **0,811** | | | 0,151 | 0,127 | -0,295 | -0,218 | | | -0,23 |
| Neck at base of fruit | **0,782** | 0,211 | -0,11 | | 0,32 | 0,335 | | | 0,161 | |
| Fruit stem and shape | **0,775** | -0,202 | | | 0,418 | | -0,189 | | | |
| Fruit pungency | **0,762** | | 0,186 | -0,507 | | | | | 0,159 | 0,121 |
| Plant height | **-0,751** | | | | | 0,112 | 0,39 | 0,158 | -0,231 | |
| Placenta size | **0,734** | 0,25 | | | | 0,424 | 0,128 | | | -0,249 |
| Stem pubescence | **0,702** | -0,422 | | 0,14 | | 0,127 | 0,327 | | -0,161 | |
| Fruit length | **-0,654** | 0,163 | -0,374 | 0,181 | 0,177 | 0,179 | | | 0,181 | |
| Fruit cross section shape | **0,614** | | | 0,225 | 0,413 | | | 0,528 | 0,13 | |
| Fruit pedicel length | **-0,596** | 0,429 | 0,112 | 0,106 | -0,149 | 0,511 | | 0,227 | | |
| Anthocyanin on hypocotyl | **0,565** | | -0,244 | | -0,318 | | | 0,454 | 0,303 | 0,26 |
| Anther color | **-0,503** | | 0,456 | -0,18 | 0,251 | 0,388 | | | 0,237 | |
| Fruit number | **-0,462** | -0,185 | 0,437 | -0,455 | | -0,216 | -0,167 | 0,192 | 0,135 | 0,327 |
| Cotyledon length | | **0,887** | 0,201 | 0,175 | | | -0,133 | -0,13 | | 0,128 |
| Leaf color | | **0,813** | | -0,324 | | -0,121 | | 0,278 | 0,123 | -0,161 |
| Leaf shape | | **-0,646** | -0,505 | -0,195 | | | 0,261 | | -0,292 | |
| Cotyledon width | | **0,622** | 0,173 | 0,417 | | | -0,349 | -0,168 | -0,195 | 0,338 |
| Fruit weight | 0,490 | **0,518** | -0,347 | -0,219 | | 0,406 | 0,156 | -0,221 | -0,159 | |
| Stem pubescence | 0,372 | **-0,394** | 0,114 | | -0,219 | 0,368 | -0,152 | | | -0,187 |
| Intensity of nodal anthocyanin | 0,329 | -0,129 | **-0,749** | 0,106 | -0,252 | | -0,117 | | | 0,306 |
| Fruit blossom end appendage | 0,344 | -0,461 | **0,657** | 0,101 | 0,153 | -0,133 | | | | 0,167 |
| Nodal anthocyanin | 0,481 | -0,276 | **-0,6** | | -0,306 | -0,115 | 0,122 | 0,126 | 0,197 | 0,282 |
| Petiole length | 0,577 | | **0,595** | -0,124 | | | 0,214 | | | 0,103 |
| Stem diameter | 0,472 | | **0,547** | -0,52 | | | 0,214 | | | |
| Fruit wall thickness | 0,352 | 0,459 | **-0,488** | -0,412 | 0,288 | | | -0,265 | | |
| Fruit surface | 0,117 | | 0,121 | **0,768** | 0,43 | | 0,136 | 0,289 | 0,13 | 0,119 |
| Yield | 0,32 | 0,393 | 0,119 | **-0,692** | | -0,186 | 0,108 | | | 0,287 |
| Stem shape | 0,235 | | 0,212 | | **-0,529** | | 0,134 | -0,152 | 0,405 | -0,161 |
| Leaf length | 0,411 | 0,212 | 0,256 | 0,516 | **-0,521** | | 0,221 | -0,146 | | 0,194 |
| Leaf width | 0,417 | 0,362 | 0,247 | 0,45 | **-0,462** | -0,145 | 0,316 | | | |
| Fruit color at green maturity | 0,374 | -0,216 | 0,446 | 0,181 | 0,156 | **0,506** | | -0,255 | -0,163 | 0,3 |
| Leaf pubescence | 0,148 | -0,12 | | | 0,574 | | **0,594** | | | |
| Plant growth habit | -0,229 | 0,374 | | 0,328 | 0,24 | -0,306 | 0,456 | -0,255 | 0,328 | -0,129 |
| Leaf margin | 0,261 | 0,373 | | | -0,16 | | 0,146 | 0,547 | -0,543 | -0,138 |

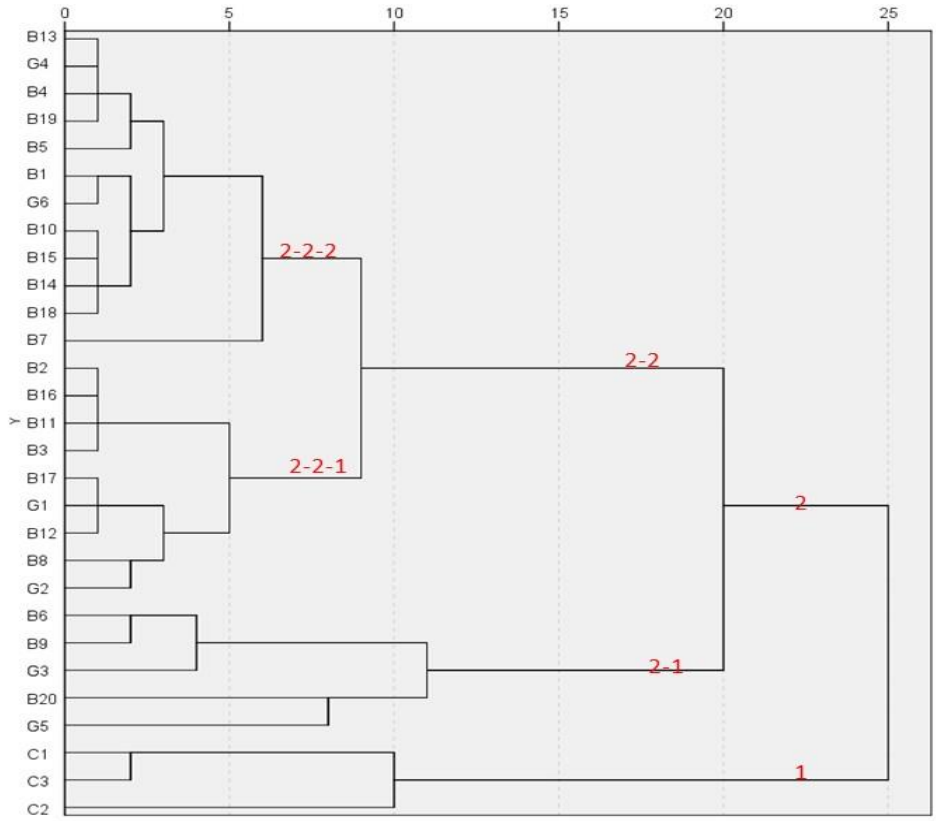


Figure 1. Grouping of 29 pepper genotypes for morphological traits in cluster analysis

## Discussion

In the exploitation of plant genetic resources (preservation and breeding), determining the existing agronomic and morphological variations within the species and revealing the distribution of this variation provide important advantages (Hawkes, 1991; İlhan, 2017). As in many plant species, the agronomic and morphological characterization of pepper genotypes obtained from different sources has been studied by many researchers (Berletti and Quagliotti, 1982; Gonzalez and Azurdia, 1985; Pentcheva, 1987; Cole, 1993; Carvalho et al., 2003; Zewdie et al., 2004; Düzyaman and Duman, 2004; Keleş, 2007; Mutlu et al., 2009; Bozokalfa and Eşiyok, 2010; Karaağaç ve Balkaya, 2010; Virga et al., 2020, Başak, 2019; Taş and Balkaya, 2021). A rich genetic diversity in plant genetic resources that form the basis of plant breeding is desirable. This genetic diversity mostly depends on the formation process of the studied plant genetic resources, the fertilization biology of the species (self-pollinated or cross-pollinated), and the diversity (climate and soil) and size of the collection areas. In accordance with the present study, variations at different rates have been reported in previous morphological characterization studies in pepper plants (Karaağaç and Balkaya, 2010; Bozokalfa and Eşiyok, 2010; Başak, 2019). In this study, among the 42 features used for morphological characterization, no variation was observed in a total of 7 features such as petal color, calyx coloration, leaf shape, and stem background color. This result was expected because the collection area was narrow, all the collected genotypes belonged to the cultivated *C. annuum* species, and the farmers exchanged seeds among themselves. Similar results regarding the aforementioned characteristics in pepper were also reported in *C. annuum* (Keleş, 2007; Binbir ve Baş, 2010; Karaağaç and Balkaya, 2010; Keleş et al., 2016; Başak, 2019). The measured characteristics showed higher variation than the observed characteristics, which supports previous studies (Bozokalfa and Eşiyok, 2010; Başak, 2019).

In the study, it was determined that fruit characteristics contributed the most to the variation and had high factor coefficients. In the first three PCs explaining 51,2% of the total variation, 19 out of 29 characteristics explaining the total variation consisted of morphological and agronomic characteristics of the fruit. Consistent with our findings, it was reported that the agronomic and morphological characteristics of the fruit contributed the most to the variation (Düzyaman and Duman, 2004; Mutlu et al., 2009; Bozokalfa and Eşiyok, 2010; Başak, 2019; Taş and Balakaya, 2021). Cross-pollination, which can sometimes reach high rates in pepper due to flower structures, and farmer selection based on fruit characteristics have a large share in the formation of high variability in fruit characteristics. Because in farmer conditions, seed production is carried out without isolation (physical or distance) and the characteristics of the male parents are ignored.

The yield of all genotypes used in the study was found to be higher than the control varieties. 13 genotypes had significantly higher yields than the average and commercial varieties. High yielding genotypes produced higher yield either by having heavier fruits or by increasing fruit number. While the high yielding genotypes B20, B9, and G3 had higher yield by having heavier fruits, G5 produced higher yield by increasing fruit numbers. These four genotypes with high yield were included in the first subgroup of the second main group in the cluster analysis (Figure 1).

Productivity in pepper is affected by traits such as plant vigor, fruit number per plant, fruit weight, and fruit flesh thickness (Arif et al., 2012). The inheritance of these characters is managed by the additive gene, dominance, and additive gene-dominance systems (Hasanuzzaman and Golam, 2011; Santos et al., 2014). In this study, high yielding genotypes produced high yields either by increasing the fruit number (G5) or weight (B20) or by increasing both (G3) (Table 5).

Characterization studies on pepper genotypes provided from different sources (collected or introduced) were carried out by various researchers. In a study by Düzyaman and Duman (2004), in which 25 different table and processing pepper genotypes were characterized in terms of 15 phenotypic characteristics, PC analysis created 4 autonomous PC axes representing 81,77% of the total variation. In the characterization study performed according to phenotype (54 traits) in 29 pepper genotypes collected from different regions of Turkey, it was determined that 9 PC's represented 85,35% of the total variation (Binbir and Baş, 2010). Karaağaç and Balkaya (2010) defined 8 groups in a clustering analysis based on 20 morphological characteristics of 56 capia pepper populations collected from Bafra. In PCA, the first three PC axes explained 74.,3% of the total variance. Variables with the highest contribution for principal component analysis were associated with plant structure and fruit descriptors in pepper and they explained 70.8% of the total variation (Villota-Cerón et al., 2012). In another characterization study conducted by Başak (2019) in 99 pepper genotypes collected from Kırşehir-Turkey region for 48 morphological traits, 75% of the total variation was explained by 17 PC's. Taş and Baklaya (2021) reported that six PC's with an Eigenvalue greater than 1 explained 70.99% of the total variation in *C. chinense*. PCA analysis is more reliable if 25% or more of the total variation in PCA analysis is explained by the first 2 or 3 axes and PC axes explain 2/3 of the total variation (Mohammadi and Prasanna, 2003; Özdamar, 2004). In the present study, 10 PC's with eigenvalues higher than 1 explained 86% of the total variation, while the first three PC's explained more than 50% of the total variation. These results show that there is significant variation between genotypes and that the study is reliable and consistent with previous studies.

## Conclusion

In this study, 26 genotypes from the Besni pepper population were characterized agronomically and morphologically, and an important variation was identified that could contribute to establishing a genetic resource for future pepper breeding studies. The presence of superior genotypes to control varieties in terms of yield and fruit characteristics indicates that there are genotypes that can be used in open-pollinated or hybrid variety breeding programs. Some of these genotypes can be used as donor or recipient parents since all of the genotypes studied are pungent and the local people prefer the hot peppers with fruit characteristics that they are familiar. To exploit genotypes that have superior characteristics in their genetic makeup in future breeding programs, pure lines should be produced either by the classical inbreeding method or by dihaploidization method. For this reason, our studies continue to determine the response of genotypes to anther culture. In addition, molecular characterization studies are being continued with different DNA markers to confirm the variation detected in the present morphological study. In today's world where local cuisines are given importance, transferring the desired characters from local genotypes to new varieties is the primary factor in ensuring the sustainability of local tastes.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Edible insect consumption and Turkish consumers' attitudes towards entomophagy

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## Abstract

Generally, in the world edible insects have gained popularity and awareness among people. Therefore, the study on Turkish consumers' attitudes and consumption towards entomophagy has important findings. The relationships edible insects with sociodemographic effects, demand to eat and personality traits of participants were determined by online questionnaire method. According to the results, most of the participants have not consumed edible insects before and furthermore both male and female consumers were not found to be positive tendency for eating edible insects. The main factors of rejection behaviors are disgusting, food neophobia and some health and religious concerns. However, a few participants have already consumed edible insects and have mainly exhibited this experience in abroad. Thereby, the study suggests that familiarity and awareness are the most significant factors changing attitudes towards to edible insect consumption for Turkish consumers. To determine consumption behavior and preferences of Turkish consumers, the most significant factors are overpassing the psychological barrier such as neophobia and facilitate entomophagy acceptance. This study could enable the development of strategies to increase desire of eating EI (edible insects) by Turkish consumers and promote insect production.

## Keywords

Entomophagy, Edible insects, Consumers' attitudes, Neophobia

## Introduction

The detrimental effects of global warming, climate change and industrial pollution cause different stress factors that affect plant survival and growth, soil properties and diversity of microbial communities (Peters, 1990). On the other hand, the global food shortage wave is likely to hit the world (Botkin et al., 2007), due to the population continues to increase in Turkey as in the world and the decrease in arable agricultural land and forests with urbanization. Therefore, alternative solutions to food sources should be developed urgently. Edible insects (EI) have great potential as an alternative, environmental friendly food source of future. Edible insects offer a sustainable solution to several problems that threaten the environment and human health, including climate change, ecological crisis and troubles from agro-industrial production.

The use of edible insects as an unconventional food source is increasing day by day for sustainability. Another reason of increasing is lack of nutritional sources such as protein, which is insufficient in some regions where food resources are becoming increasingly decrease (Flachowsky et al., 2017). In addition, edible insects, which are a new product for people's seeking for new taste and flavor, contain many nutrients such as protein, fatty acids, vitamins, and minerals which necessary for a healthy life (Rumpold and Schlüter, 2013). In addition, edible insects require less water and space requirements and cause lower greenhouse gas emissions during production. Realizing the production of edible insects with more sustainable characteristics towards the environment, they are more effective at

changing digested matter to biomass compared to conventional livestock.

Most insect species which used in insect production have high amount of protein, fatty acids, fiber, vitamins and minerals (Belluco et al., 2013; Finke and Oonincx, 2014). The chemical composition of insects differs depending on the species and genus, diet, period of growth (Ramos-Elorduy, 2002; Oonincx and Van Der Poel, 2011) and the habitat in which natural environment of the insects live. In addition, the nutritional composition of insects is probable to be affected by the preparation methods such as frying, boiling, baking, grinding and drying for consumption. Although the preparation methods and insect characteristics are different, insects are an unconventional source of quality animal protein (Verkerk et al., 2007; Srivastava et al., 2009). Although the edible insect consume risks in terms of food safety and health are not clear, edible insects are consumed as a nourishing and pleasant-tasting food source in a great number of societies of the world due to differences in nutritional value (Verkerk et al., 2007; Srivastava et al., 2009; Klunder et al., 2012; Imathiu, 2020).

Entomophagy is a term that consuming insects as food and it includes about 1500-2000 known species, in Asia, Africa, Australia and United States of America. The most common types of EI are Coleoptera (31%), Lepidoptera (18%), Hymenoptera (14%), Orthoptera (13%), Hemiptera (10%), Isoptera (3%), Odonata ( 3%) , Diptera (2%) and others (5%) (Van Huis et al., 2013). In European countries, although consumer acceptance is low, its awareness and preference is becoming more common.

According to studies, the main reason of low preference to consume in European countries is food neophobia which is related to human behaviors such as sources of disgust (consumption of insects, abnormal fear, bad odors) (Gere et al., 2017; Piha et al., 2018; Mancini et al., 2019; Lammers et al., 2019; Orkusz et al., 2020; Florença et al., 2021; Detilleux et al., 2021). The neophobic consumers have a tendency to avoid and reluctant to try new foods as similar to children's reluctance to eat novel food. However this behavior might be change, for example, edible insects can be integrated in different dishes such as meat products, sauces, bakery products, cookies and protein bars (Homann et al., 2017; Piha et al., 2018; Orkusz et al., 2020; Detilleux et al., 2021).

Considering the articles have been published on the opinions of Turkish consumers regarding edible insect, in literature there is a few numbers of research on the term of entomophagy in the Turkey (Karaman and Bozok, 2019; Kaymaz and Ulema, 2020). The goal of the current study is to investigate knowledge and attitude of the respondents in Turkey about potential of insects and insect-based products or dishes.

Within this framework, we get to know about the impact of gender and sociodemographic factors on attitudes towards edible insects as a food product, to identify the factors that prevent the consumption of edible insects and the reasons for motivation to consume of edible insects. Moreover, it is planned the outcomes of this study contribute to the existing literature on edible insect consumption.

## Materials and Methods

### Data collection and sampling

In this study, an online questionnaire was used to analyze for determination of Turkish consumers' attitudes for edible insect consumption. The data collection was widely disseminated nationwide in October 2021 via email, social media platforms and personal references. Respondents willingly filled out the questionnaire and were not given any incentives.

### Questionnaire design

The survey consisted of 18 questions and questionnaire design was divided into the four parts, regarding about demographics, general knowledge about entomophagy, desire to consume and consumption experience (Hartmann et al., 2015; Barsics et al., 2017). Sociodemographic questions consisted of question on gender, age, residence, education level, occupation and income.

For general knowledge about entomophagy, participants answered the following the questions; "'Have you ever previously heard of the term entomophagy? if you heard, where had you learned about entomophagy?', 'Do you aware of any benefits of EI?' and 'Have you eaten EI before?'". In third part of questionnaire, questions about the desire to consume were asked to participants such as their opinion on the desire, factors that prevent the consumption of EI and demand for different forms of edible insects. Finally in the last part, participants, who had tasted, responded questions concerning; the consumption place(s) of edible insects, entomophagy experience, the form of consumed insects and consumption frequency.

### Statistical analysis

The results of questionnaire about edible insect consumption and determination of Turkish consumers' attitudes were performed by using statistical software SPSS V22 (SPSS Inc., Chicago, IL, USA). Basic descriptive statistics was used to summarize findings by describing the relationship between variables in the study. In addition, a crosstab tool combined with the chi-square test was used to evaluate whether there were significant relationships between some of the categorical variables examined considering the 5% significance level. Determining to the effects on edible insect consumption, a P-value of $< 0.05$ was considered statistically significant.

## Results and Discussion

### Demographic features

A total of 427 fully filled questionnaires, thought to represent the population in Turkey, were collected. Concerning sociodemographic variables of participants, the sample consisted of 52.9% men and 47.1% women, which relates to the sex distribution in Turkey (50.1% male; 49.9% female). The all respondents were living in Turkey and over the age of fifteen. Age of the respondents ranged from 18 to 88, and more than half of the participants were under age 35 (51.1%). Respondents were from 39 cities placed every region in Turkey and the most was from İstanbul (65.2%). Majority of the respondents had a high education level (Bachelor's degree and higher 56.4%) and working in different areas (Table 1). The annual income of is classified by proportioning the annual net amount of the

2021 minimum wage in Turkey (Ministry of Labor and Social Security).

Table 1. Sociodemographic features of the participants

| Sociodemographic features | n | Percent (%) |
|---|---|---|
| Gender | | |
| Male | 226 | 52.9 |
| Female | 201 | 47.1 |
| Age | | |
| 18–25 years | 116 | 27.2 |
| 26–35 years | 102 | 23.9 |
| 35< years | 209 | 48.9 |
| Education level | | |
| High school or below | 48 | 11.2 |
| University student | 92 | 21.5 |
| Associate degree | 46 | 10.8 |
| Bachelor's degree and higher | 241 | 56.4 |
| Annual Income | | |
| Under 34.000TL | 131 | 30.7 |
| 34.000TL-47.999TL | 53 | 12.4 |
| 48.000TL-71.999TL | 96 | 22.5 |
| 72.000TL-120.000TL | 90 | 21.1 |
| Over 120.000TL | 57 | 13.3 |
| Occupation | | |
| Student | 192 | 45.0 |
| Food staff | 44 | 10.3 |
| Others | 191 | 44.7 |

## General knowledge about entomophagy

After the demographic questions, knowledge levels and attitudes of participants about entomophagy were measured (Table 2). According to results, most of the participants (62.3%) had not heard of the term entomophagy and 40.5% of participants gained familiarity of entomophagy via this study. Although 58.1% of the participants are not aware of the benefits of insect consumption (Table 2), participants who have knowledge about the insect consumption, consider insects as a cheap and nutritious food source (Figure 1).

Table 2. General knowledge about entomophagy of the participants

| Variables | n | Percent (%) |
|---|---|---|
| Hearing of entomophagy | | |
| Yes | 161 | 37.7 |
| No | 266 | 62.3 |
| Where was learned | | |
| Family/Friends | 25 | 5.9 |
| School | 27 | 6.3 |
| Restaurant | 4 | 0.9 |
| TV/Books/News | 95 | 22.2 |
| Internet/Social media | 79 | 18.5 |
| Travel abroad | 24 | 5.6 |
| In this study | 173 | 40.5 |
| Behavior | | |
| Beneficial | 175 | 41 |
| Ineffective | 4 | 0.9 |
| No idea | 248 | 58.1 |

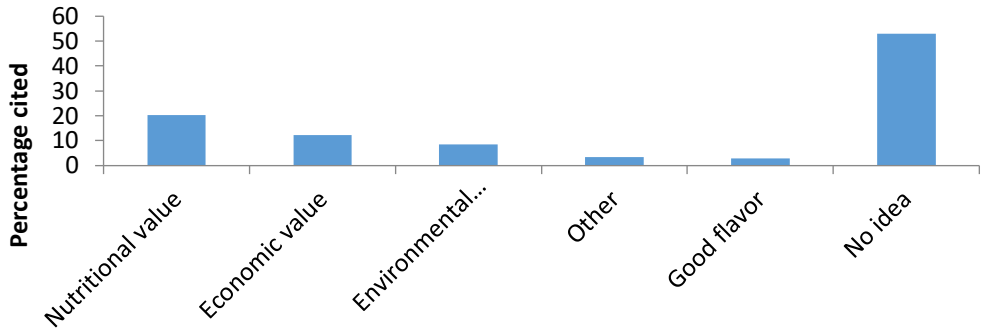


Figure 1. Considered benefits of edible insect production and consumption by participants

About 94.1% of participants (n=402) had not consumed edible insects (Table 3) and of these, 79.6% have significantly unwilling to consume ($p<0.05$). The relationship between sociodemographic variables (gender, age, education level, annual income, and occupation) and willing to consume edible insects was not statistically significant (respectively $\chi^2 = 1.725$. P =0.631; $\chi^2 = 22.953$. P =0.001; $\chi^2 = 24.615$. P =0.003; $\chi^2 = 20.139$. P =0.065). Although, the most of participants (78.6%) do not want to try and to consume edible insects whatever form it has, a little part of participants (14.1%), who have willing to try the edible insects, want to consume in invisible form ($p<0.05$).

Table 3. Willing to edible insects of the participants

| Variables | n | Percent (%) |
|---|---|---|
| Consuming edible insects | | |
| Yes | 25 | 5.9 |
| No | 402 | 94.1 |
| Willing to consume (n:402) | | |
| Unwilling | 340 | 79.6[a] |
| Willing | 53 | 12.4[b] |
| Undecided | 9 | 2.1 |
| Desired consumption form (n:402) | | |
| Visible | 29[a] | 7.2[a] |
| Invisible | 57[b] | 14.1[b] |
| Unwilling | 316 | 78.6 |

Different letters in the table means with differ significantly ($p<0.05$).

According to respondents, the major factors influencing consumption of edible insects were determined as being disgusting (Figure 2). Therefore, more than half of the respondents refused eating EI as food (54.7%). Consistently, health and religious concerns (29.2%) and unfamiliar (8.1%) were the other reasons for refusal of EI consumption in their diets. Bad flavor (3.6%), negative environmental impact (1.9%), low availability (1.3%) and social/cultural pressure (1.2%) were stated as another reason. Despite a growing awareness of nutritional and environmental benefits, desires of EI consumption do not increase similarly for majority of the population. This phenomenon has been explained as a consequence of the psychological barriers of consumers.

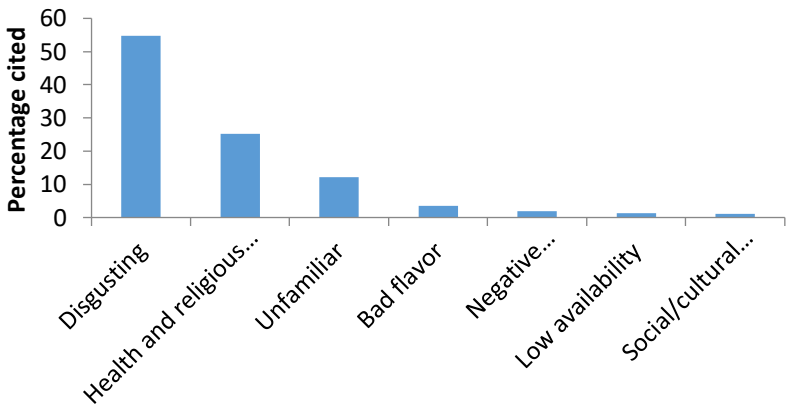


Figure 2. Factors influencing consumption of edible insects

According to the findings, only 5.9% of respondents (n=25) had eaten edible insects before this study (Table 3). Most of the Turkish experienced respondents (76%) have tried edible insects once, during traveling abroad (84.0%) ($p<0.05$) and have approximately 80.0% satisfied with this experience. Moreover, more than half

of the experienced participants (52%) expressed their appearance as neutral. Overall, Turkish consumers showed a neutral attitude towards eating again, buying and consuming regularly edible insects. Therefore, the production and sale of edible insects remains limited in Turkey. Form of edible insects was found as a factor affecting consumption frequency and willing for consumption. The form of edible insects influences consumers' willing. About 40.0% of respondents, who consumed before, consumed visible type of insects and 60.0% of respondents who consumed before, consumed foods containing invisible insect ingredients.

Table 4. Consumption experience of the participants at study (n=25)

| Variables | N | Percent (%) |
|---|---|---|
| Entomophagy experience | | |
| Bad | 5 | 20.0[b] |
| Neutral | 13 | 52.0[a] |
| Good | 7 | 28.0[b] |
| Consumption place | | |
| Travel abroad | 21 | 84.0[a] |
| Local restaurant | 4 | 16.0[b] |
| Form of consumed insects | | |
| Visible | 10 | 40.0 |
| Invisible | 15 | 60.0 |
| Consumption frequency | | |
| Tried it once | 19 | 76.0 |
| Tried it more than 2 times | 5 | 20.0 |
| Occasionally/regularly | 1 | 4.0 |

Different letters in the table means with differ significantly ($p<0.05$).

Evaluating the awareness of Turkish consumers is crucial to understand their preference of EI consumption. The attitude for EI consumption is desired to be consumed more by men compared to women in America (Woolf et al., 2019), but in Turkey, the significant difference in EI consumption was not found between the gender in this study ($p > 0.05$).

Previous researches, in Uganda (Olum et al., 2021), Italy (Palmieri et al., 2019) and Germany (Hartmann et al., 2015; Lammers et al., 2019) show that consumers' education level and familiarity are affected on consumption of edible insects. Moreover, edible insects are frequently consumed in Asia (Mitsuhashi, 1997; Hanboonsong et al., 2013; Yen, 2015; Liu et al., 2019) and Africa (Hlongwane et al., 2020). However, according to results; edible insects are not preferred by Turkish consumers of all ages and segments. These differences could be probably related to wide range of products and dishes in Turkish cuisine arise from varying climate due to geographical location of Turkey.

Researches in the field of edible insect consumption show that the factors of low tendency towards edible insects are food neophobia, insect phobia and disgusting (Liu et al., 2019; Sogari et al., 2019; Toti et al., 2020; Olum et al., 2021), In this study, although the main factors of rejection behaviors are disgusting and food neophobia, religious and health concerns are other factors influencing consumption of edible insects.

The main reason for this attitude stems from the unfamiliarity and lack of knowledge of consumers about edible insects. Moreover, consumers are very little aware of the term of entomophagy, but the major group have become aware of it by this study and have offered thank for the study. Therefore, it can be stated that the study increased awareness on the term of entomophagy. Additionally, the participants who more familiar and informed about entomophagy, they have felt more willing to consume EI ($p < 0.05$).

Another finding that supports a significant contribution to familiarity is that only 5.9% of Turkish participants consume EI in the present study, while 88% of respondents in the Kenyan consumer consume EI (Pambo et al., 2016). The cause of this ratio may be that the awareness and consumption of entomophagy is higher in other countries such as Belgium and Kenya. These results give a lead about increasing the consumption of EI as the societies are informed about the term of entomophagy. In addition, the briefing sessions has been presented to develop willingness to consume EI (Barsics et al., 2017). This study also agrees with the findings of Pambo et al. (2016), who reported that EI consumption is positively affected by increased knowledge of entomophagy.

More than half of the respondents currently do not want to consume EI due to the "disgust" factor. Studies have shown that the disgust response in societies is learned at a young age and is generally passed down through generations (La Barbera et al., 2018; Woolf et al., 2019). In order to prevent this, information on edible insects should be included in primary school education, websites, and nutritional advice (Van Huis., 2016). Furthermore, informing parents will increase their children's familiarity with edible insects and will affect their consumer approach in the future (Looy et al., 2014).

## Conclusion

Edible insects are consumed by societies in the world as an integral part of gastronomic culture. For some time, EI has been discussed and surfaced as a sustainable food source, as the world population has continued and will continue to increase at a rate incompatible with the current state of Earth resources. Edible insects have been shown to have great potential at both the nutritional and environmental levels.

The main purpose of this work is to determine Turkish consumers' attitudes towards entomophagy and

remove the barriers production and consumption of edible insects. Therefore, this study is specially considered that how some factors such as demographic, familiarity, knowledge of entomophagy and concerns affect to decision of Turkish consumers to consume edible insects.

In this study has shown the results from a sample of Turkish consumers' regarding knowledge, attitude and degree of acceptability of EI consumption. It was revealed that the majority of the respondents who were participated to survey did not consume edible insects at all, and those who did mostly consumed it abroad.

Based on these findings, for possible introduction of EI into the Turkish food market should start by foods that incorporate EI such as invisible forms can be recommended. Additionally, although people in Turkey seem aware of the implications of consuming EI at the sustainability level, concerning their nutritional and health effects, the Turkish consumers do not want to consume edible insects due to the food neophobia and some health and religious concerns. Therefore, it is still necessary to raise the dissemination of information about the advantages and/or limitations of edible insects.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Ethical approval for the study was obtained from Istanbul Rumeli University (Decision No: 9, Protocol No: 2021/08, Date: 13.10.2021).

Not applicable.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Evaluation of performance indicators for some drip irrigation systems used in cherry orchards in Ankara province

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## Abstract
In this study, performance tests of drip irrigation systems were carried out in cherry orchards of some farmers in Ankara. Accordingly, measurements were made on the day of irrigation in the lateral and manifold pipes of the drip irrigation systems used by the farmers. Within the scope of the research, Distribution Uniformity, Emission Uniformity, Application Efficiency, Potential and Actual Application Efficiency measurements were made in drip irrigation systems in 10 different cherry orchards. For this purpose, 4 different dripper points were determined on at least 4 lateral lines in each manifold line. Each lateral pipe and dripper point was chosen to be at the beginning of the line, 1/3 of the line, 2/3 of the distance and at the end of the line. Measurements were made on farmers' irrigation days and under farmer conditions. According to the obtained results, the CU value was between 77.5%- 93.0%, DU value between 60.1%- 86.9%, EU value between 56.7%- 84.5% and CV value between 0.09- 0.28. As a result of the research, it has been determined that there are important design and application problems in drip irrigation systems.

## Keywords
Cherry orchard, Water management, Irrigation performance, Water distribution

## Introduction
Due to the increasing population, the demand for food is constantly increasing. To meet the increasing food demand, more agricultural production and more agricultural irrigation are required. However, due to climate change and the increase in water use in other sectors, irrigation water resources in agriculture are gradually decreasing. This situation requires more efficient use of water in agriculture. 74% of water resources in Turkey are used in agriculture. This rate is well above the European average and it is predicted that the water rate in the agriculture sector will decrease to 64% in the near future (Anonymous, 2022). Despite the decrease in the amount of water to be used in agricultural irrigation, water resources should be used with high efficiency by using pressurized irrigation systems for sustainable production. The use of pressurized irrigation systems should be increased in order to irrigate more agricultural areas with less water in agriculture. With the decrease in the costs of pressurized irrigation systems in the near future, it is expected that a significant part of the irrigated agricultural lands will be irrigated with pressurized systems.

One of the most important ways to save water in agriculture is to use pressurized irrigation systems. (Ibragimov et al., 2007; Darouich et al., 2014; Qureshi

et al., 2015; Gültekin and Ertek 2018). It is also important to operate the pressurized irrigation system correctly, to distribute the water homogeneously in the field, to meet the plant water requirement on time and at an optimum level. The benefits of pressurized irrigation systems have been demonstrated in many studies carried out under controlled trial conditions (Woltering et al., 2011; Tagar et al., 2012; Yan et al., 2018; Fan et al., 2020). However, the use of pressurized irrigation systems in agriculture cannot guarantee the effective use of water resources. The maintenance needs of the irrigation system and the competencies of the users are also very important. Especially in smallholders, pressurized irrigation systems are managed as a continuation of old habits. This situation may prevent the potential of the system to be utilized sufficiently and may cause excessive or insufficient irrigation. This situation may prevent the potential of the system to be utilized sufficiently and may cause excessive or insufficient irrigation. In addition, since the control unit elements (filter, manometer, fertilizer tank, etc.) that should be used in the irrigation system are often neglected, water application efficiency decreases and the economic life of the irrigation system may decrease. Considering the decrease in water resources and the high costs of pressurized irrigation systems, it is clear that the system should be well planned and supervised in terms of engineering.

In this study, the performance tests of drip irrigation systems in cherry orchards were measured under farmer conditions. Accordingly, some performance parameters (irrigation efficiency, water uniformity, etc.) were measured after the design, application and technical examination of the drip irrigation system of 10 different farmers.

## Material and Method

### Experimental site

The research was conducted in 2018 in Ayaş district of Ankara, located in the Central Anatolia Region, longitude 40.01° N and latitude 32.19° E. Typical features of the continental climate are observed, with cold winters and hot and dry summers. The altitude is 910 meters, the annual average temperature is 11.4 Cº, the average relative humidity is 54%, and the annual average rainfall is 439.7 mm (Anonymous, 2021a). The most cherry production in Ankara is made in Ayaş district. In addition, intensive vegetable and fruit production is carried out in the region (Anonymous 2021b).

Some physical and chemical properties of the soils of the trial plots were analyzed. For this purpose, disturbed and undisturbed soil samples were taken from soil profiles (0-0.3, 0.3-0.6, 0.6-0.9 and 0.9-1.2 m). the soil texture of the study area was generally clayey and the water used in irrigation was suitable for irrigation in terms of irrigation water quality.

### Measurements and analysis

In the tested drip irrigation systems, 4 different laterals on each secondary pipe (manifold) were selected. These were the first laterals on the manifold, at 1/3 and 2/3 distances from the beginning of the manifold and last laterals at the end of the manifold. Selected emitters were first or second dripper at the entrance of the lateral, at the distances of 1/3, 2/3 of the lateral and at the end of the lateral. Thus, 16 test points were selected in each sub-unit (Figure 1).

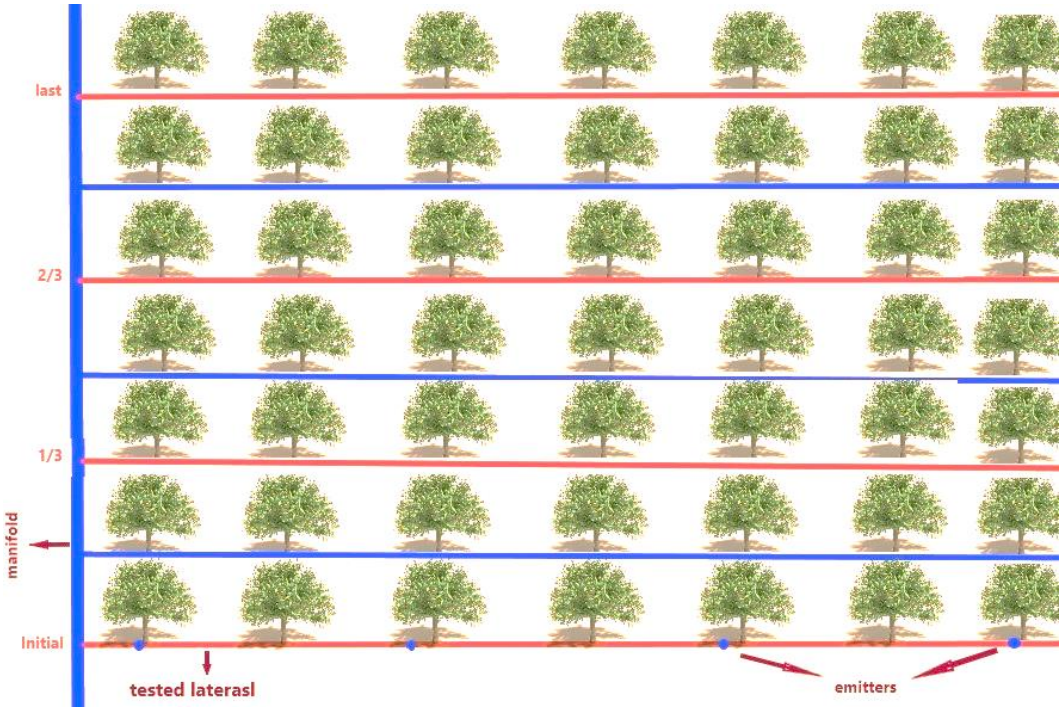


Figure 1. Schematic representation of the measurement locations made in the sub-unit of the drip irrigation system.

The flow rates of the drippers at the test points on the laterals were measured volumetrically. Each dripper flow was measured 5 times; the average flow rate of a dripper was determined. According to field measurements, the following performance parameters were calculated (ASAE, 1991 ve 1998; Kanber et al., 1996; Burt et al., 1997; Ortega et al., 2002).

### Coefficient of variation (Cv)

$$Cv = \frac{Sd}{qa} \quad (1)$$

$$Sd = \frac{\sqrt{((q1^2 + q2^2 + \ldots\ldots + qn^2 - nqa^2)/(n-1))}}{qa} \quad (2)$$

$$qa = \frac{1}{n}\sum_{n=1}^{n} q_i \quad (3)$$

Where; *Sd*: Standard deviation; *qa*: Average flow rate of tested drippers *(l h$^{-1}$); q1, q2 …… qn*: flow rates of the drippers tested. In point source drippers, dripper flow rate variation coefficient (Cv) is classified as Cv <0.05 very good, Cv = 0.05-0.07 medium, Cv = 0.07-0.11 at the border, Cv = 0.11-0.15 bad and CV> 0.15 unacceptable. (ASAE, 1998).

**Uniformity Coefficient (CU)**

Uniformity coefficient (Christiansens, 1942) was measured according to equation as follows:

$$CU = 100\left(1.00 - \frac{\sum_{i=1}^{n}|(q_i - \overline{q})|}{\overline{q} \times n}\right) \quad (4)$$

Where; *n*, number of observations or number of drippers used in evaluation; $q_i$ dripper flow, l h$^{-1}$; $\overline{q}$, average dripper flow, l h$^{-1}$.

**Distribution Uniformity (DU)**

Distribution Uniformity was determined as another index of application co-distribution. It was calculated as the ratio of the average value (sub quarter average flow rate) of the lowest 1/4 of the emitter flow rates considered in the evaluated sub-unit to the average flow rate for the sub-unit (James 1988; Kanber et al., 1996).

$$DU = 100\frac{\overline{q_{lq}}}{\overline{q}} \quad (5)$$

In the equation, $\overline{q_{lq}}$ refer to the lower quarter average emitter flow rate, l h$^{-1}$, , $\overline{q}$ indicates the average emitter flow, l h$^{-1}$.

**Emission Uniformity (EU)**

It was determined using the approach given by Keller and Karmeli (1974). For this purpose, the equation below was used.

$$EU = \left[1 - 1.27\frac{Cv}{N^{0.5}}\right]\frac{q_{min}}{\overline{q}} \quad (6)$$

where *N*: number of drippers evaluated; $q_{min}$, minimum emitter flow rate, l h$^{-1}$; $\overline{q}$, average emitter flow rate, l h$^{-1}$.

**Statistical Uniformity (Us)**

It was calculated using the equation given below according to the principles given by Bralts and Kesner (1983).

$$Us = 100\ (1 - C_v) = 100\ \left(1 - \frac{S_d}{\overline{q}}\right) \quad (7)$$

**Absolute Emission Uniformity (Eua)**

It was calculated with the equation suggested by Sivanappan and Padmakumari (1980).

$$E_{ua} = \left(\frac{100}{2}\right)\left(\frac{Qn}{Qa} + \frac{Qa}{Qx}\right) \quad (8)$$

Where, *Qn*= minimum emitter discharge in the subunit, l h$^{-1}$, *Qa* = average emitter discharge in the subunit, l h$^{-1}$, *Qx* = maximum emitter discharge in the subunit, l h$^{-1}$

**Application Efficiency (Ea)**

It was determined according to the following equation according to ASAE (1991 and 1998) and Kanber et al (1996).

$$Ea = 100\ \left[Vr\ \frac{1-Pd}{Va}\right] \quad (9)$$

In the equation, *Vr*, the required amount of water, m$^3$; *Va*, the total amount of water applied, m$^3$; (1-Pd), irrigated root zone,% (percentage of wetting); *Pd*, unwetted area,%; *Qa*, Actual flow rate of irrigation system, m$^3$ h$^{-1}$; T, irrigation time, h.

**Potential Application Efficiency of Lower Quarter (PELQ) and Actual Application Efficency of Lower Quarter (AELQ)**

It was determined using the approaches given by Merriam et al. (1980).

$$PELQ = 100\ \left(\frac{MAD}{\overline{d}}\right) \quad (10)$$

$$\overline{d} = \overline{q} \times T \times \frac{1}{A} \quad (11)$$

$$AELQ = 100\ \frac{SMD}{\overline{d}} \quad (12)$$

Where: MAD, the amount of water allowed to be consumed or the amount of moisture consumed in the soil, mm; $\overline{d}$, the average depth of water applied, mm; T, irrigation time, h; A, area wetted by drippers, m$^{2.}$; the SMD indicates the missing moisture amount, mm, at the desired soil depth to be irrigated.

**Wetting Pattern (P)**

The wetting pattern was determined by measuring the width of the wet strip that occurs along with the lateral(s) of the land surface 24 hours after irrigation. The wetting rate was calculated using the equation given below.

$$P = 100\ \frac{A_w}{S_a \times S_w} \quad (13)$$

$$A_w = W \times S_w \quad (14)$$

Where, $A_w$ is the wetted area, m$^2$; P is wetting percent, %; $S_a$, tree row spacing, m; $S_w$, tree-to-row distance, m; W is the width of the wet strip, m;

**Storage Efficiency (Es)**

The equation given by James (1988) and Kanber et al. (1996) was used in the calculations.

$$E_s = 100 \times \frac{S_{rz}}{SMD} \quad (15)$$

In the equation, $S_{rz}$ is the amount of water stored in

the root zone (or soil depth to be wetted) during irrigation, mm; SMD, the amount of water missing in the root zone before irrigation (the amount of water required to bring the current humidity to field capacity), mm.

### Maximum Irrigation Depth (Imax)

It was calculated using the following equation according to ASAE (1991 and 1998).

$$\text{Imax} = y\,(AW)\,\frac{Z.P}{100} \quad (16)$$

In the equation, y is the amount of water allowed to be consumed before irrigation, %; AW, available water capacity, mm/m; Z is soil to be wetted or root depth, m; P, wetting percent, %

### Emitter Performance Coefficient of Variation ($V_{pf}$)

The emitter performance variation coefficient was determined using the equation specified in ASAE (1991).

$$V_{pf} = \left(C_v{}^2 - V_{qh}{}^2\right)^{1/2} \quad (17)$$

$V_{pf}$ (%) Classification was made according to ASAE (2003). Accordingly; $V_{pf}$ > 0.20 unacceptable, 0.15–0.20 poor, 0.10–0.15 acceptable, 0.05–0.10 good, < 0.05 excellent

### Emitter Discharge Coefficient of Variation Due to Hydraulics ($V_{qh}$)

This performance measure was determined using the following approach described in ASAE (1991).

$$V_{qh} = X \times V_{hs} \quad (18)$$

where $X$ is the emitter discharge exponent and $Vhs$; hydraulic design coefficient of variation were determined using equations 1, 3 and 4 respectively.

Drip systems having good emission uniformity indicate that water and injected fertilizer are distributed evenly throughout the cherry orchards. Emission Uniformity Rating was defined as 90 - 100% Excellent, 80 - 90% Good 70 - 80% Fair, < 70% Poor

### Results and Discussion

The irrigation water analyses used by farmers in the research are given in Table 1.

Well water was used for irrigation in all plots where the studies were carried out. The PH values of the irrigation water varied between 7.09-7.51 and EC values between 0.59-4.33 dS $m^{-1}$. While there was no problem in terms of alkalinity in irrigation water, high salinity ($T_4A_1$) was determined in 1 irrigation water.

Soil samples were taken at 0-0.3, 0.3-0.6, 0.6-0.9, and 0.9-1.2 m depths of the soil to evaluate the performance of drip irrigation systems (Table 2). The characteristics, irrigation numbers, and irrigation times of the drippers used in the trial plots are shown in Table 3.

In the examined test plots; soil structure was generally clay and loamy-clay structure, infiltration rate (I) varied between 0.9-7.7 mm $h^{-1}$. Organic matter amounts were between 0.22-1.75%, lime amounts were between 3.5-53.4%. The number of irrigation in cherry orchards was between 2-5, the irrigation duration was between 8-24 hours. Irrigation practices varied according to the habits of the farmer, the capacity of the water source, and the size of the irrigated area. It was observed that the soils on which the tests were carried out generally had a clayey texture and were poor in organic matter. Accordingly, it was determined that the water uptake rate of the soil was generally low. It has been determined that the runoff, which is frequently seen in the test areas, is caused by the long irrigation time and high flow drippers.

### Dripper Flow Rates and Dripper Pressures

The measured average dripper flow rates and dripper pressures are given in Table 4.

Table 1. Irrigation water analysis results

| Test Farmer No | Water Source | pH | EC dS $m^{-1}$ | SAR | Irrigation water class |
|---|---|---|---|---|---|
| $T_1$ | Deep Well | 7.51 | 0.76 | 0.88 | $T_3A_1$ |
| $T_2$ | Deep Well | 7.09 | 0.59 | 0.69 | $T_2A_1$ |
| $T_3$ | Deep Well | 7.30 | 0.64 | 1.21 | $T_2A_1$ |
| $T_4$ | Deep Well | 7.47 | 1.75 | 0.72 | $T_3A_1$ |
| $T_5$ | Deep Well | 7.45 | 4.33 | 0.84 | $T_4A_1$ |
| $T_6$ | Deep Well | 7.37 | 1.12 | 1.56 | $T_3A_1$ |
| $T_7$ | Deep Well | 7.32 | 1.00 | 1.90 | $T_3A_1$ |
| $T_8$ | Deep Well | 7.39 | 0.82 | 1.40 | $T_3A_1$ |
| $T_9$ | Deep Well | 7.30 | 0.64 | 1.21 | $T_2A_1$ |
| $T_{10}$ | Deep Well | 7.22 | 2.00 | 0.58 | $T_3A_1$ |

Table 2. Soil properties of the examined test plots

| No | Depth (cm) | Bulk density (gr $cm^{-3}$) | Field capacity | Fading point | Structure | Infiltration (mm $h^{-1}$) | EC (dS $m^{-1}$) | PH | Organic Matter (%) | Lime Amount (%) (Çağlar, 1949) |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 0-0.3 | 1.23 | 27.08 | 20.3 | CL | 1.5 | 1.14 | 8.0 | 1.75 | 10.1 |
| | 0.3-0.6 | 1.17 | 36.72 | 19.0 | C | | 1.44 | 7.4 | 1.00 | 9.5 |
| | 0.6-0.9 | 1.21 | 36.82 | 19.4 | C | | 1.31 | 7.4 | 0.85 | 10.0 |
| | 0.9-1.2 | 1.18 | 40.22 | 19.9 | C | | 1.06 | 7.4 | 0.79 | 11.4 |
| $T_2$ | 0-0.3 | 1.26 | 27.55 | 15.2 | SCL | 7.7 | 0.5 | 7.5 | 0.94 | 8.0 |
| | 0.3-0.6 | 1.22 | 28.80 | 16.7 | CL | | 0.39 | 7.6 | 0.51 | 6.3 |
| | 0.6-0.9 | 1.24 | 28.56 | 14.2 | CL | | 0.49 | 8.0 | 0.49 | 9.0 |
| | 0.9-1.2 | 1.33 | 24.7 | 14.9 | L | | 0.44 | 8.0 | 0.41 | 18.0 |
| $T_3$ | 0-0.3 | 1.2 | 36.72 | 19.0 | C | 4.6 | 0.48 | 7.4 | 1.20 | 7.4 |
| | 0.3-0.6 | 1.2 | 40.34 | 19.9 | C | | 0.6 | 7.8 | 1.09 | 7.0 |
| | 0.6-0.9 | 1.28 | 38.87 | 20.2 | C | | 0.49 | 7.9 | 0.87 | 8.2 |
| | 0.9-1.2 | 1.25 | 37.15 | 21.3 | C | | 0.41 | 7.7 | 0.77 | 8.7 |
| $T_4$ | 0-0.3 | 1.27 | 32.23 | 16.77 | SCL | 8.4 | 0.56 | 8.0 | 1.20 | 21.0 |
| | 0.3-0.6 | 1.28 | 27.93 | 13.66 | SCL | | 0.37 | 8.1 | 0.44 | 21.8 |
| | 0.6-0.9 | 1.24 | 27.65 | 12.8 | SCL | | 0.35 | 8.1 | 0.36 | 19.7 |
| | 0.9-1.2 | 1.32 | 25.33 | 14.89 | SCL | | 0.56 | 8.0 | 0.22 | 22.5 |
| $T_5$ | 0-0.3 | 1.13 | 48.25 | 25.54 | C | 4.2 | 0.71 | 8.0 | 1.33 | 33.1 |
| | 0.3-0.6 | 1.27 | 29.03 | 15.41 | CL | | 0.88 | 8.1 | 0.95 | 41.4 |
| | 0.6-0.9 | 1.24 | 32.12 | 16.85 | CL | | 0.85 | 8.1 | 0.90 | 44.6 |
| | 0.9-1.2 | 1.21 | 32.18 | 15.18 | CL | | 0.98 | 8.1 | 0.67 | 48.7 |
| $T_6$ | 0-0.3 | 1.13 | 48.25 | 25.54 | C | 1.2 | 0.7 | 8.2 | 1.36 | 33.8 |
| | 0.3-0.6 | 1.27 | 29.03 | 15.41 | CL | | 0.9 | 8.3 | 0.97 | 42.2 |
| | 0.6-0.9 | 1.24 | 32.12 | 16.85 | CL | | 0.9 | 8.2 | 0.92 | 45.5 |
| | 0.9-1.2 | 1.21 | 32.18 | 15.18 | CL | | 1.0 | 8.2 | 0.68 | 49.7 |
| $T_7$ | 0-0.3 | 1.16 | 36.57 | 20.56 | C | 5.1 | 0.64 | 8.0 | 1.42 | 5.5 |
| | 0.3-0.6 | 1.27 | 37.39 | 20.16 | C | | 0.55 | 7.8 | 0.48 | 7.2 |
| | 0.6-0.9 | 1.26 | 40.38 | 20.1 | C | | 0.64 | 7.7 | 0.54 | 6.9 |
| | 0.9-1.2 | 1.28 | 38.92 | 20.2 | C | | 0.56 | 7.9 | 0.36 | 7.9 |
| $T_8$ | 0-0.3 | 1.15 | 50.09 | 20.44 | C | 0.9 | 0.52 | 8.0 | 0.67 | 8.9 |
| | 0.3-0.6 | 1.13 | 49.13 | 20.13 | C | | 0.37 | 8.1 | 0.50 | 5.5 |
| | 0.6-0.9 | 1.17 | 42.45 | 19.28 | C | | 0.42 | 8.2 | 0.43 | 3.5 |
| | 0.9-1.2 | 1.24 | 40.42 | 20.92 | C | | 0.46 | 8.1 | 0.33 | 5.4 |
| $T_9$ | 0-0.3 | 1.19 | 37.34 | 19.07 | C | 1.4 | 0.46 | 8.1 | 1.49 | 14.4 |
| | 0.3-0.6 | 1.18 | 37.42 | 18.8 | C | | 0.49 | 8.0 | 0.84 | 25.2 |
| | 0.6-0.9 | 1.29 | 37.54 | 16.57 | C | | 0.54 | 7.9 | 0.64 | 27.2 |
| | 0.9-1.2 | 1.23 | 45.84 | 21.4 | C | | 0.61 | 8.0 | 0.66 | 27.7 |
| $T_{10}$ | 0-0.3 | 1.19 | 34.89 | 22.15 | C | 1.7 | 0.45 | 7.4 | 1.17 | 39.7 |
| | 0.3-0.6 | 1.18 | 37.08 | 18.91 | C | | 0.61 | 7.3 | 0.86 | 51.8 |
| | 0.6-0.9 | 1.29 | 40.36 | 27.13 | C | | 1.39 | 7.6 | 0.73 | 53.4 |
| | 0.9-1.2 | 1.23 | 35.42 | 21.42 | C | | 1.15 | 8.0 | 0.95 | 48.1 |

Table 3. Features of drippers used in test plots, number of irrigation and irrigation durations

| | Emitter flow | Emitter range | Num. of irrigation per season | Avr. irrigation duration |
|---|---|---|---|---|
| | (L $h^{-1}$) | (m) | | (h) |
| **$T_1$** | 4 | 0.20 | 3 | 12 |
| **$T_2$** | 4 | 0.25 | 3 | 12 |
| **$T_3$** | 4 | 0.20 | 3 | 8 |
| **$T_4$** | 4 | 0.25 | 5 | 12 |
| **$T_5$** | 4 | 0.25 | 4 | 8 |
| **$T_6$** | 4 | 0.25 | 3 | 10 |
| **$T_7$** | 4 | 0.33 | 4 | 12 |
| **$T_8$** | 4 | 0.25 | 4 | 24 |
| **$T_9$** | 4 | 0.33 | 4 | 12 |
| **$T_{10}$** | 4 | 0.25 | 2 | 24 |

Table 4. Average dripper flow rates and dripper pressures

| Test no | Natural flow rate of the dripper, (q) | Measured actual emitter flow rate, ($q_{avr}$) | Average emitter pressure, ($h_{avr}$) |
|---|---|---|---|
| | (L $h^{-1}$) | (L $h^{-1}$) | (atm) |
| **$T_1$** | 4 | 2.30 | 0.42 |
| **$T_2$** | 4 | 2.41 | 0.32 |
| **$T_3$** | 4 | 5.58 | 0.42 |
| **$T_4$** | 4 | 3.01 | 0.56 |
| **$T_5$** | 4 | 5.08 | 0.37 |
| **$T_6$** | 4 | 4.53 | 0.56 |
| **$T_7$** | 4 | 5.00 | 0.33 |
| **$T_8$** | 4 | 2.35 | 0.61 |
| **$T_9$** | 4 | 4.65 | 0.52 |
| **$T_{10}$** | 4 | 1.83 | 0.33 |

In the study, the average emitter flow rates were measured between 1. 83-5.58 l $h^{-1}$. Average dripper pressures varied between 0.32-0.61 atm. Dripper pressures were measured below the accepted operating pressure (1 atm) in all plots. In heavy textured soils with low infiltration rates, high dripper flow rates may cause surface flow. On the other hand, the clogging problem is more common as the flow rate decreases. Therefore, the operating pressure should not be less than 1 atm (Yıldırım and Korukçu, 1999). Accordingly, while it was determined that some drippers had a much higher flow rate due to production, it was determined that some drippers operated with a low flow rate due to clogging or low pressure.

### Emitter Flow Coefficient of variation (Cv)

The dripper flow coefficient of variation (Cv) for the tested parcels varied between 0.09 and 0.28 and their classifications was made according to ASAE (1998) and given in Table 5. Cv values were determined as at the limit in 1 plot, and unacceptable in the others. In parcels classified as unacceptable, dripper flow rates varied in a wide range.

Most of the drip irrigation systems used in the cherry orchards where the research was conducted were older than 4-5 years. In the research, it has been determined that the periodic maintenance of drip irrigation systems is generally ignored by the farmers. Therefore, CV values of irrigation systems in all test areas were found to be insufficient. The Cv values obtained in the study were higher than Camp et al. (1997); Gil et al. (2008) and Elamin et al. (2017).

### Evaluations of water distribution and water use efficiency

Some parameters showing the efficiency and suitability of drip irrigation systems used in cherry orchards were calculated (Table 6).

Table 5. Evaluation of emitter flow rate variation coefficients, (ASAE, 1998)

| Test no | Cv | Classification |
|---|---|---|
| **$T_1$** | 0.09 | at the limit (0.07-0.11) |
| **$T_2$** | 0.28 | Unacceptable (>0.15) |
| **$T_3$** | 0.17 | Unacceptable (>0.15) |
| **$T_4$** | 0.21 | Unacceptable (>0.15) |
| **$T_5$** | 0.16 | Unacceptable (>0.15) |
| **$T_6$** | 0.18 | Unacceptable (>0.15) |
| **$T_7$** | 0.17 | Unacceptable (>0.15) |
| **$T_8$** | 0.20 | Unacceptable (>0.15) |
| **$T_9$** | 0.21 | Unacceptable (>0.15) |
| **$T_{10}$** | 0.21 | Unacceptable (>0.15) |

Table 6. Some performance indicators of drip irrigation systems

| Test no | CU, % | DU, % | EU, % | Us, % | Eua, % | Ea, % | PELQ, % | AELQ, % | $I_{max}$, mm | Es, % | P, % | Vpf, % | Vqh, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 93.0 | 86.9 | 84.5 | 91.2 | 95.8 | 74.1 | 76.0 | 74.1 | 20.3 | 55.9 | 21.2 | 0.08 | 0.11 |
| $T_2$ | 77.5 | 70.6 | 64.2 | 71.8 | 100.0 | 71.4 | 57.8 | 71.4 | 17.3 | 91.3 | 22.6 | 0.27 | 0.04 |
| $T_3$ | 86.5 | 72.2 | 66.2 | 83.1 | 97.7 | 83.2 | 59.6 | 87.8 | 25.1 | 133.2 | 18.8 | 0.14 | 0.60 |
| $T_4$ | 82.9 | 73.0 | 68.1 | 78.7 | 98.9 | 80.2 | 61.2 | 80.2 | 17.4 | 88.4 | 18.8 | 0.12 | 0.09 |
| $T_5$ | 87.4 | 81.7 | 76.7 | 84.2 | 96.6 | 92.2 | 69.0 | 92.2 | 16.7 | 91.1 | 10.6 | 0.15 | 0.17 |
| $T_6$ | 85.4 | 72.8 | 68.6 | 81.7 | 97.7 | 66.6 | 61.7 | 66.6 | 17.5 | 68.0 | 15.8 | 0.14 | 0.03 |
| $T_7$ | 86.1 | 60.1 | 56.7 | 82.7 | 99.8 | 88.8 | 51.1 | 88.8 | 28.5 | 94.0 | 22.6 | 0.17 | 0.11 |
| $T_8$ | 84.4 | 73.4 | 67.9 | 80.4 | 98.6 | 73.8 | 61.1 | 73.8 | 26.6 | 145.7 | 13.6 | 0.18 | 0.04 |
| $T_9$ | 82.9 | 69.3 | 64.1 | 78.6 | 98.7 | 75.8 | 57.7 | 75.8 | 21.8 | 117.5 | 16.1 | 0.20 | 0.07 |
| $T_{10}$ | 83.1 | 75.4 | 69.1 | 78.9 | 98.0 | 70.1 | 62.2 | 69.1 | 19.3 | 136.6 | 20.4 | 0.20 | 0.08 |

CU values of the test plots varied between 77.5-93.0%. In the tests, it was determined that the CU values were generally acceptable. Obtained CU values showed similarities with Safi et al. (2007); Ella et al. (2009); Elamin et al. (2017) at similar operating pressures, but lower than Jamrey and Nigam (2018)

As an indicator of uniformity, the DU is a very sensitive parameter and takes into account only the lowest quarter of the observed discharges in relation to the average discharge. This is different from the coefficient of uniformity, which takes into account all observed discharge values in relation to the mean.

In the study, DU values varied between 60.1-86.9%, as expected, DU values were found to be relatively lower than CU. Accordingly, only two of the irrigation systems were rated as "good" and the others had lower performance values. In the study, lower DU values were obtained than Camp et al. (1997); Jamrey and Nigam (2018); and higher than Ella et al. (2009). When the results of the study were evaluated, the slope of the land and the pressure changes in the irrigation system were seen as important factors on DU.

In the results obtained, the EU values of the test plots varied between 56.7-84.5%. According to evaluation criteria, EU values were within the recommended limit values in the $T_1$ test and below the limit values in other tests. In the study, lower EU values were obtained than Camp et al. (1997); Jamrey and Nigam (2018); Uygan and Çetin (2015) and higher than Ella et al. (2009).

In the research, while Us values varied between 71.8-91.2%, Eua values varied between 95.8-100%. In terms of US, the measurement made on only one test was evaluated as "good". On the other hand, Eua values were good and above in all tests. Accordingly, it has been determined that the irrigation systems were suitable for absolute emission uniformity. Eua values were similar or higher than most research findings (Noori and Al Thamiry, 2012; Mistry et al. 2017; Abdulhadi and Alwan 2020).

Ea is an indicator of how well irrigation water has been applied. In the study, Ea value was determined to be "good" and above in only two tests. This means that in most tests the irrigation was insufficient or the root zone of the plant was not sufficiently wetted.

PELQ is a measure of how well the system performs when optimum water is applied in the plant root zone and AELQ is an indicator of operation and management status in irrigation systems (Bhavan and Maro, 1991). For systems, Potential Application Efficiency (PELQ) in the lower quartile ranged from 51.1-76.0%, and Actual Application Efficiency of the Lower Quarter (AELQ) ranged from 66.6-92.2%. The AELQ values obtained in the study were found to be similar to Uygan and Çetin (2015) and higher than Zare et al., (2020), the PELQ values were found to be lower than Uygan and Çetin (2015) and higher than Zare et al., (2020). The smaller the difference between AELQ and PELQ, the better the system operates (Ashiri et al., 2014). It will be seen that this difference is not low in all drip irrigation systems examined. In this case, it can be said that the tested drip irrigation systems are not well operated or not properly designed.

The maximum irrigation depth ($I_{max}$) is used to calculate the application efficiency and is converted to the net irrigation requirement when multiplied by the irrigated area. In the tests performed, the $I_{max}$ value varied between 17.3 and 28.5. Obtained Imax values were found suitable according to the soil types (Hezarjaribi, 2008).

The storage efficiency (Es) indicates how well the system uses the available root zone storage capacity to store water to meet crop needs. Es values in the tested areas varied between 55.9-145.7%. An Es value of more than 100 indicates excessive irrigation in the root zone of the plant, while values below 100 indicate insufficient water needs (Anyoji and Wu, 1994; Irmak et al. 2011).

In drip irrigation system planning, it is extremely important to determine the wetting area percentage (P) correctly. This rate generally varies between 30-70% of the total area, especially in orchards. For this reason, the percentage of the wetting area should be at least 30% in project designs. However, this value can be taken as the lower limit of 25% in humid regions and 35% in very arid regions (Keller and Bliesner, 1990). Wetting percentages (P) in the tested plots were found to be between 10.6% and 22.6%. The reason for the wetting area ratio to vary in such a wide range was due to the different tree-planting spacings, the differences in the lateral spacing, and the differences in the amount of irrigation water applied. The fact that the wetting rate was lower than 30% in all irrigation systems where the tests were carried out showed that the system design and irrigation practices were faulty.

Emitter performance coefficient of variation ($V_{pf}$) is a measure of dripper flow variability due to dripper wear, clogging, water temperature and dripper construction characteristics. In previous similar studies, it was reported that $V_{pf}$ values were between 0.027 and 0.275 and $V_{qh}$ values were between 0.019-0.047 (Camp et al. 1997; Safi et al. 2007). The $V_{pf}$ values obtained in the study ranged from 0.08 to 0.27. Emitter discharge coefficient of variation due to hydraulics ($V_{qh}$) values were between 0.03-0.17. Accordingly, 4 of the tested systems were within the acceptable limit, while the others were below the acceptable limit. According to these findings, it was determined that there was a problem in terms of pressure homogeneity and hydraulic uniformity in the system.

## Conclusion

The drip irrigation system is one of the most effective irrigation methods that saves water. Dripper technologies, which are continuously developed to increase irrigation efficiency, and soil and plant moisture monitoring devices provide a great convenience for users. The infiltration properties of the soil, plant water requirement, the quality of the material used, climatic characteristics of the region, user's knowledge level and habits may affect the performance of the irrigation system.

In this study, the performance tests of drip irrigation systems in cherry orchards belonging to farmers were measured. It has been tried to demonstrate how effectively drip irrigation systems are used without interfering with the irrigation habits of the farmers. According to the findings obtained in the study, CU values in drip irrigation systems were generally found to be appropriate, DU values were low except for 2 parcels, especially because the sloping structure of the land was ignored. System performances were sufficient in terms of absolute emission uniformity. However, only one parcel was sufficient for Us and Eu. In the study, it was seen that there was sufficient irrigation application in only 2 test plots. The difference between AELQ and PELQ was huge. This indicates that the drip irrigation system is not well operated or well designed. In the observations made in the cherry orchards, it was seen that the drip irrigation systems were installed on the land by the farmers and they did not have any technical knowledge. Therefore, the design of the system was poor. Es values were very high especially due to dripper flow rates that are not compatible with the infiltration rate of the soil and very long irrigation time. This situation causes water losses by deep infiltration or surface run-off. Wetting rates of the irrigation system were also below 30% in all systems. This is technically undesirable. However, wetting rates were found to be low due to the fact that farmers generally used only one lateral. Irrigation systems tested for Vpf and Vqh were generally at or below the limit value. Pressures at the dripper point varied considerably, as the operating pressure of the system was generally low.

The effectiveness of pressure irrigation systems in conserving water largely depends on the habits and skills of the users. This study showed that user-related problems (design, operation) are very important in drip irrigation system performance. For this reason, it is necessary to benefit from engineers who are experts in irrigation in the stages of designing the drip irrigation system and applying it to the field.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Ethics committee approval is not required.

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### Consent for publication

Not applicable.

### Data Availability Statement

No data, models, or code were generated or used during the study.

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# Investigation on the effects of cooking methods on anti-inflammatory and antioxidant activities of five mostly consumed vegetables in winter

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## Abstract

The aim of this study is to investigate the effects of steaming and boiling on antioxidant and anti-inflammatory activity of five frequently consumed vegetables in winter. The vegetables were prepared by three different cooking methods including steaming, 5-minute boiling, 15-minute boiling to compare with their raw forms. Antioxidant capacity was measured with 1, 1-diphenyl–2 picrylhydrazyl (DPPH) radical scavenging method, anti-inflammatory activity was measured with 5-lipoxgenase inhibitory method, and the total phenolic content was detected after *in vitro* cooking process. The highest antioxidant activities among the raw vegetables were found in spinach (SR) (425.80 µg/mL, 12.83 mg/g) and broccoli (BR) (754.50 µg/mL, 7 mg/g). While boiling for 15 minutes and steaming slightly increased the antioxidant capacity in spinach and decreased it in broccoli; the highest activities among the mentioned cooking methods and vegetable samples were detected in these two vegetables. The lowest antioxidant activities were determined in leek (LR) (5662.0 µg/mL, 1.24 mg/g) and celery (CeR) (2796.0 µg/mL, 2.98 mg/g). In addition, the least affected vegetable from the cooking methods was spinach (S) (227.4-549.8 µg/mL). Cooking techniques have significative effects on the levels of phytochemical compounds and antioxidant capacities. It was observed that cooking methods increased the anti-inflammatory activity of the vegetables used in this study. Only broccoli (B) showed a decrease. The highest anti-inflammatory activity was found in the leek (L15) which was boiled for 15 minutes. Also, our results contribute to the databases that provide information about the effects of different cooking techniques on the antioxidant and anti-inflammatory potential of vegetables.

## Keywords

Anti-inflammatory activity, Antioxidant activity, Cooking method, DPPH, Vegetable

## Introduction

The concept of sustainable healthy life has gained importance with the changes in lifestyle in recent years. Consumption of healthy foods is the main component of a healthy lifestyle (Mori et al., 2009). Since growing evidence suggested the effect of nutrition on the healthy and long life, especially in recent years, the antioxidant intake has gained great importance in the developed countries (Finley et al., 2011). The consumption of fruits and vegetables significantly reduced the incidence of various chronic diseases including hypertension, cancer, stroke, and many others. Phytochemical compounds such as vitamins, flavonoids, and phenolic compounds are assumed to contribute to these health benefits (Grebmer et al., 2014; Grosso et al., 2013).

Green-leafy vegetables contain various types of active ingredients, which possess antioxidant activity. The majority of these active ingredients are vitamins and minerals, in addition to non-nutritive compounds such as quercetin. Overall, these active molecules play significant role in preventing diseases such as cancer (Wach et al., 2007; Abuajah et al., 2015).

Most vegetables are commonly cooked before being consumed in the food service industries, and homes. Cooking is known to cause some changes in the chemical composition through altering the

bioavailability and concentration of phytochemical compounds such as vitamin C, carotenoids and polyphenols (Pellegrini et al., 2010).

The cooking process has been used since centuries to increase the flavor and extend the shelf life. However, high heat treatments often cause changes in physicochemical properties of the phytochemical compounds (Kosewski et al., 2018). Based on this, it is concluded that the phytochemical compositions of the vegetables change during heat treatment. Vegetables are usually consumed after being cooked in water or oil and at various temperatures or time periods. Therefore, cooking is a significant process that directly affects the final product obtained from vegetables (Soares et al., 2017; Miglio et al., 2008). Morales and Babel (2002) proposed four possible mechanisms to understand the reasons behind the change in the antioxidant activity of vegetables upon heat treatment: 1) release of higher amounts of antioxidant compounds due to the destruction of cell walls and cellular compartments, 2) formation of the new antioxidants or compounds 3) suppression of the antioxidants through thermal inactivation of oxidative enzymes, 4) production of the active radical scavengers.

Although the common sense suggests that the vegetables should be consumed unprocessed and preferably uncooked, there is growing evidence showing that the absorption of many nutrients is increased by cooking. While pharmacological effects of some vegetables are more prominent upon raw consumption, the others require appropriate cooking methods to boost the effect. However, further studies focusing on the effect of cooking on the nutritional content of vegetables remain needed (Link and Potter, 2004; Miglio et al., 2008). The effect of different cooking techniques on the organoleptic properties and phytochemical compound of vegetables have been investigated in several studies. Yet, different findings have been observed due to the processing conditions and the type of samples (Dolinsky et al., 2016; Iborra-Bernad et al., 2015; Palermo et al., 2014).

Vegetables contain a wide range of hydrophilic and lipophilic antioxidant compounds. Therefore, antioxidant activity should be evaluated by different methods since these compounds function synergistically and might stop free radicals in both phases (Abuajah et al., 2015).

Although there are various analytical methods developed to determine the antioxidant activity, using different methods and complicated systems, these methods are still in the development phase. While chemical principles are valid in these methods, an antioxidant shows high antioxidant activity with the selected measurement method, while the same antioxidant may show a lower activity with another method. Therefore, it is recommended to work with at least two methods to determine the antioxidant capacity and compare them (Aruoma, 2003).

Thus, the aim of this study is to establish a relationship among the type of cooking methods (raw, steamed, boiled), time of processing (raw, 5-minute boiling, 15-minute boiling, 5-minute steaming), the antioxidant capacity of five most consumed vegetables (broccoli, cabbage, celery, leek and spinach) in winter (Onur et al., 2017). It has been stated that the antioxidant activity is associated with the total phenolic content (Aruoma, 2003). Therefore, while selecting the method, TPC was performed in addition to DPPH measurement and it was aimed to determine whether the antioxidant activity was correlated with the anti-inflammatory (5-lipoxygenase inhibition) activity.

## Materials and Methods

### DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical scavenging activity assay

DPPH radical scavenging activity was determined as described along with slight modifications (Brand-Williams et al., 1995). Shortly, the stock extracts were prepared in MeOH as 10 mg/mL concentrations and diluted to 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL with MeOH. 10 µL of each dilution was mixed with 190 µL of 0.1 mM DPPH radical solution (Sigma-Aldrich) prepared in MeOH and each dilution was loaded into 96 well-plates, in triplicates. The plates were shaken gently and left in the room temperature and dark for 40 minutes until the measurements. Afterwards, absorbances were measured at 517 nm (Synergy HTX Biotech multimode ELISA reader). The half inhibitory concentration ($IC_{50}$) values, which show the concentration that caused a 50% inhibition of radical formation, were used to clarify the results. Gallic acid was used as the positive control. All tests were performed in triplicate.

### Anti-inflammatory activity

5-lipoxygenase inhibition activity was performed according to the method suggested by Phosrithong and Nuchtavorn (Phosrithong and Nuchtavorn, 2016) with slight modifications described by Yıldırım et al. (Yıldırım et al., 2019). 10 µL of the extracts or standard indomethacin were added to 20 µL ethanol, 25 µL of sodium borate buffer solution (pH 9, 0.1 M), 20 µL pure water, and 25 µL of type V soybean lipoxygenase solution in the buffer (pH 9, 20.000 U/mL). The reaction mixture was pre-incubated for 5 minutes at 25 °C. Then, 100 µL of 0.6 mM linoleic acid solution was added to the solutions, mixed well and the change in absorbance at 234 nm was followed for 6 min. Each reaction was run for three times.

### Determination of Total Phenolic Content (TPC)

Total phenolic contents in all extracts were established by Folin-Ciocalteu (FC) assay (Singleton and Rossi, 1965). Briefly, 5 µL from the test samples (10, 5, 2.5 and 1.25 mg/mL in MeOH) were mixed with 25 µL 0.2 N FC reagent and 145 µL distilled water the mixture were shaken quietly. Following six minutes incubation, 75 µL of $Na_2CO_3$ (7%) solution was added and shaken again. The plates were incubated at dark for two hours and at the room temperature. Absorbances at 765 nm were measured. Gallic acid was used as the standard. All tests were performed in triplicate.

### Statistical Analysis

The data were given as means±standard deviations and statistically analyzed using one-way ANOVA followed by the Tukey's test. Multiple Comparison test with the confidence interval (CI) of 95% for each was performed using GraphPad Prism 5. Differences between means at $p<0.05$ levels were considered as statistically significant.

## Results and Discussion

Many vegetables are consumed raw or cooked. Various cooking methods are applicable, but the most common ones for vegetables are steaming and boiling.

The antioxidant and the anti-inflammatory activity, also the total phenolic contents, results of the samples determined according to each cooking method were shown in Table 1.

Various evaluations were made according to these results. Vegetables with the highest antioxidant capacity were determined. The effect of the cooking method applied to each vegetable on antioxidant/anti-inflammatory activity and TPC were evaluated (Table 1).

The highest antioxidant activities and the total phenolic contents among raw vegetables were spinach (SR) (425.80 µg/mL, 12,83 mg/g) and broccoli (BR) (754.50 µg/mL, 7 mg/g). While boiling for 15 min and steaming slightly increased the antioxidant capacity in spinach, it decreased in broccoli. The highest activities among the mentioned cooking methods and raw vegetable samples were found in these two vegetables (Table 1).

The lowest antioxidant activities and the total phenolic contents among raw vegetables were determined in leek (LR) (5662.0 µg/mL, 1.24 mg/g) and celery (CeR) (2796.0 µg/mL, 2.98 mg/g). When cooking methods were compared, steaming the broccoli, cabbage and spinach; 15-minute boiling celery and leek were found to have the highest antioxidant activities. In addition, the vegetable that was the least affected by these cooking methods was spinach (S) (227.4-549.8 µg/mL) (Table 1).

It can be said that antioxidant activity is correlated with the total phenolic contents. It was also found that steaming increased TPC in broccoli (BV) and spinach (SV). Boiling process (5 minutes or 15 minutes) reduced TPC in broccoli (B), cabbage (C) and spinach (S). In other samples, TPC slightly increased or does not change with the boiling process (Table 1).

The highest antioxidant activity among all raw samples was observed in spinach (S). Similarly, the highest antioxidant activity among all cooked samples was detected in spinach (S) as well. When raw and boiled samples were compared, the antioxidant activity and TPC decreased in broccoli and cabbage upon boiling, while it increased in leek. When all samples were evaluated in terms of antioxidant activity, the vegetables with the highest antioxidant activity were found to be spinach (S) (227.4-549.8 µg/mL) and broccoli (B) (523.9-927.2 µg/mL) (Table 1).

There are other studies investigating the effect of steaming and boiling. Turkmen et al. (2005) studied the effect of cooking on the antioxidant activity of broccoli and found an increase after boiling, microwaving and steaming. In our study, the antioxidant effect of broccoli decreased by cooking. Paciulli et al. (2018) worked with brussels sprouts and zucchini to achieve more preferable physical (texture, color) and antioxidant results compared to steaming. Their results showed that there was a linear change in force/compression firmness for brussels sprouts and an increase in total phenolic content for zucchini when cooking time and/or temperature were changed. Steamed brussels sprouts showed significantly higher antioxidant activity values than air-steaming or raw. Miglio et al. (2008) investigated the effects of boiling and steaming on the phytochemical compounds and antioxidant activity of broccoli. The results showed an increased antioxidant activity upon steam cooking, possibly due to increased phytochemical compounds than the other cooking methods. An overall increase in antioxidant activity values were observed in all samples, possibly due to matrix softening and increased compounds that can be converted into new antioxidants. Also, in our results, DPPH values increased in boiling cabbage (C15) and broccoli (B15) for 15 minutes and decreased in celery (Ce15), leek (L15) and spinach (S15). In steaming, the DPPH values increased in broccoli (BV), spinach (SV), leek (LV) and celery (CeV), whereas decreased in cabbage (C). Similar to our results, Perez-Burillo et al. (2019) founded a relation among the type of processing, time of processing, antioxidant capacity, and the development of the Maillard reaction of 23 widely consumed vegetables. They determined that, by density, normal and well-cooked samples showed significantly higher antioxidant capacities than raw ones. In our samples, the activity increased in celery (Ce), leek (L) and spinach (S), yet decreased in cabbage (C) and broccoli (B) with the cooking process. The cooking process, the appliances and the tools used in our study have critical differences compared to the previous research. Additionally, the cooking time and other phytochemical componds found in the vegetables are known to influence the outcome. It is also seen that many other vegetables not included in our study are also affected by the cooking processes. Also, these differences in results can be explained by the change in the structure or concentration of phytochemical compounds with the cooking process.

In addition, conditions such as climate, growing and storage affect the chemical composition of the vegetable and thus its activity (Mazzeo et al., 2011).

Steaming or boiling can increase the antioxidant capacity of green leafy vegetables. In our results, the highest antioxidant activities of all raw vegetables were found in spinach (S) detected by DPPH. In addition, spinach was the least affected vegetable by the cooking processes. In a study, Hossein et al. also investigated the effect of different cooking methods on antioxidant capacity. They selected three spinach species and green leaved amaranth which are antioxidant rich. The results showed a significant increase in TPC, total flavonoid content (TFC) and reducing power (RP) in all samples. These results reveal the effects of different heat treatments on the antioxidant potential of vegetables. In raw, green leaf amaranth, water spinach leaf, and Indian spinach leaf had similar TPC, while garden spinach had the lowest. Both boiling and frying significantly increased the radical scavenging ability of DPPH compared to the raw ones (Hossain et al., 2017). As in their study, decreases were also found using the radical scavenging ability of DPPH by Martínez-Hernández et al. (2013) in boiled and steamed broccoli. Ramirez-Anaya et al. (2015) also determined the fat, moisture, TPC, eighteen phenolic compounds and antioxidant capacities of raw vegetables and compared them with the values obtained after cooking. Ultimately, antioxidant capacity measured by all cooking methods

remained constant or increased, however TPC decreased. Compared with our findings, different results were obtained in the vegetables we studied, as seen in Table 1. While the antioxidant activity increased in some samples, the activity decreased in others after cooking.

Results from previous studies, which evaluated the effect of temperature on the TPC of vegetables are contradictory. Adefegha and Oboh (2011) found that steaming caused a significant increase in TPC and TFC of eight tropical leafy greens upon cooking. This is thought to be related to the breakdown of cell walls and the release of bioactive compounds. Conversely, Lafarga et al. (2018) investigated the antioxidant potential of some Brassica vegetables and the effect of steaming and sous-vide treatments on TPC. In most of the evaluated samples, no difference was found between phenolic compounds loss after steaming and sous-vide. Dolinsky et al. (2016) evaluated the effects of different cooking techniques on the polyphenols and antioxidants of vegetables. Zucchini, collard greens, cabbage, green beans, broccoli, carrots and tomatoes were subjected to different cooking techniques and compared with their raw forms. The results showed that the heat treatment had a significant effect on the antioxidant capacity and soluble polyphenolic content. They recommended steaming as the best cooking method for these vegetables to increase the level of both antioxidants and polyphenols. In accordance with these, in our results, steaming increased TPC in spinach (S) and broccoli (B), in which the greatest increase was observed in spinach (from 12.83 mg/g to 20.47 mg/g). At the same time, the increase in TPC is higher in the 15-minute boiling method compared to 5-minute boiling except leek (L).

It was observed that cooking methods increased the anti-inflammatory activity of the vegetables used in this study. Only broccoli showed a decrease. The highest anti-inflammatory activity was found in the leek (L15) which was boiled for 15 minutes. However, cabbage (C15), celery (Ce15) and spinach (S15) boiled for 15 minutes showed strong activity (respectively 0.05 mg/mL, 0.06 mg/mL and 0.07 mg/mL)

It is seen that the five vegetables used in this study are affected differently by the cooking processes. (Table 1)

Table 1. The antioxidant activity, the anti-inflammatory activity and the total phenolic content results

| Samples | Method | Raw (R) | Boiling 5 min. (5) | Boiling 15 min. (15) | Steaming (V) |
|---|---|---|---|---|---|
| | | $IC_{50}$, µg/mL | | | |
| B | DPPH | 754.5±1.56$^{b}$ | 927.2±2.33$^{d}$ | 794.7±3.18$^{c}$ | 523.9±0.42$^{a}$ |
| | Anti-lipoxgenase | 0.18±0.00$^{a}$ | 0.42±0.00$^{b}$ | 0.39±0.00$^{b}$ | 0.17±0.04$^{a}$ |
| | TPC (mg/g) | 7.0±0.04$^{bc}$ | 4.99±0.06$^{a}$ | 6.91±0.09$^{b}$ | 8.43±0.19$^{c}$ |
| C | DPPH | 1421±14.85$^{a}$ | 4218±2.83$^{d}$ | 2240±34.65$^{c}$ | 1694±9.19$^{b}$ |
| | Anti-lipoxgenase | 0.60±0.00$^{c}$ | 0.15±0.00$^{a}$ | 0.05±0.00$^{a}$ | 0.38±0.00$^{b}$ |
| | TPC (mg/g) | 3.29±0.03$^{c}$ | 1.08±0.03$^{a}$ | 2.05±0.06$^{b}$ | 2.07±0.01$^{b}$ |
| Ce | DPPH | 2796±9.90$^{c}$ | 1481±9.90$^{ab}$ | 1129±22.63$^{a}$ | 1805±6.36$^{b}$ |
| | Anti-lipoxgenase | 0.59±0.00$^{d}$ | 0.24±0.00$^{b}$ | 0.06±0.00$^{a}$ | 0.54±0.01$^{c}$ |
| | TPC (mg/g) | 2.98±0.04$^{b}$ | 1.96±0.00$^{a}$ | 3.48±0.04$^{c}$ | 2.93±0.23$^{b}$ |
| L | DPPH | 5662±37.48$^{d}$ | 2077±6.36$^{b}$ | 1469±18.38$^{a}$ | 3493±0.71$^{c}$ |
| | Anti-lipoxgenase | 0.65±0.00$^{c}$ | 0.30±0.00$^{b}$ | 0.04±0.00$^{a}$ | 0.47±0.01$^{bc}$ |
| | TPC (mg/g) | 1.24±0$^{ab}$ | 2.30±0.03$^{c}$ | 1.40±0.04$^{b}$ | 0.81±0.04$^{a}$ |
| S | DPPH | 425.80±0.99$^{c}$ | 549.80±6.72$^{d}$ | 314.50±2.83$^{b}$ | 227.40±2.69$^{a}$ |
| | Anti-lipoxgenase | 0.05±0.00$^{a}$ | 0.16±0.00$^{b}$ | 0.07±0.01$^{a}$ | 0.28±0.00$^{c}$ |
| | TPC (mg/g) | 12.83±0.04$^{b}$ | 7.66±0.06$^{a}$ | 13.58±0.06$^{b}$ | 20.47±0.04$^{c}$ |
| Control | Gallic acid (DPPH) | 2.46±0.01 | | | |
| | Indomethacine | 0.02±0.00 | | | |

*Abbreviations: B, C, Ce, L and S show methanol extract obtained from vegetables; raw (R), boiling 5 minutes (5), boiling 15 minutes (15) and steaming (V) fractions. **Each value in the table is represented as mean ± SD. Different letter superscripts in the same line indicate significant differences ($p<0.05$).

## Conclusion

The vegetables are significant part of nutrition and they are consumed either raw or cooked. The evident suggests that the preferred cooking method alters the antioxidant activity, which has critical roles in preventing diseases. Therefore, the importance of the cooking method elevates. In our study, the antioxidant activity increased in Ce, L and S samples upon cooking, yet decreased in B and C samples. Additionally, boiling time was detected to affect the activity and TPC. For example, the antioxidant activity of sample L was found to increase when boiled for 15 minutes compared to 5-minute boiling. In contrast, boiling spinach for 15 minutes boiling decreased the antioxidant activity compared to other cooking methods. Altogether, it is concluded that each vegetable was affected differently from the cooking processes. Our findings have identified that antioxidant activity is closely related to total phenolic content. Boiling methods and less preferred steaming methods are effective in increasing the antioxidant capacity of vegetables. The total increase in TPC values observed in this study partially contradicts the claim that cooked vegetables have lower nutritional value than raw ones. Heat treatment has a decisive effect on the bioactive component and antioxidants of vegetables. Our results also contribute to a database that provides information about the effects of different cooking methods on the antioxidant capacity of vegetables. It is important to provide enlightening information to the consumers and people who are responsible for the preparation and cooking of foods, especially by investigating the effects of home cooking methods on nutritive value. Additionally, considering the complex chemical matrix of the vegetables, further studies are needed to investigate the effect of cooking methods of vegetables on health.

### Preparation of samples

Broccoli (*Brassica oleracea* var. *botrytis*), celery (*Apium graveolens*), cabbage (*Brassica oleracea* var. *capitata*), leek (*Allium ampeloprasum*) and spinach (*Spinacia oleracea*) were taken from a local market. Broccoli (B), cabbage (C), celery (Ce), leek (L) and spinach (S) were washed. Then, the vegetables were dried, 100 grams of them were weighed and they were prepared based on three different cooking methods.

Raw: 100 g of samples were cut into small pieces and placed in containers to be processed (BR, CR, CeR, LR, SR).

Boiling (5 minutes-15 minutes): 100 g of samples were added to 200 ml of boiled tap water and left to boil for five minutes in a covered stainless-steel cooker. For the 15 minutes boiling group, the same procedures were repeated and applied for 15 minutes. Afterwards, samples were filtered and allowed to cool (B5, C5, Ce5, L5, S5 and B15, C15, Ce15, L15, S15).

Steaming: 100 g of samples were added to sieves, which were placed on top of pots with 150 ml of boiled water. Samples cooked in the steam of the water for five minutes were removed and allowed to cool (BV, CV, CeV, LV, SV).

### Extraction procedure

All samples were cut into small pieces by a blender. Samples were left to macerate for 48 hours by shaking occasionally in 200 ml methanol in the dark. After 48 hours later, the extracts were filtered. Then methanol was evaporated using a rotary evaporator. The extracts were kept at +4 °C until the time of use.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Ethical approval

Ethics committee approval is not required.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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# Pitfalls and prospects of contract farming to smallholder tobacco farmers in Shamva district, Zimbabwe

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## Abstract
The objective was to assess the challenges and opportunities of contract farming to smallholder tobacco farmers in ward 27, Shamva District, Zimbabwe. Data on demographics, household endowments and economic and social achievements from tobacco production was collected using questionnaires and interviews. A systematic sampling technique was used in selecting the tobacco farmers. Data was analysed using SPSS version 26. The results show that 93.9% of the participants were males and most of the participants were educated to primary level (36.4%). Zimbabwe Leaf Tobacco Company (43%) was cited as the major company which provide contract farming to tobacco farmers in ward 27. The results further indicated that there are more opportunities received by tobacco farmers such as receiving inputs at no cost but face challenges such as poor crop yields due to low producer price (55%), high cost of production (30%), low rainfall (9%) and shortage of firewood (6%). Some participants indicated that they do not have technical knowledge on how to use chemicals and in identifying pests so that they apply the correct chemicals. It is recommended that companies which provide contract farming must collaborate with the Ministry of Agriculture and further train farmers on financial management skills, how to use chemicals and pest identification so that the quality of the leaf is improved. Nevertheless, contracting companies should provide tillage services to farmers and ensure that farmers are included in price determination.

## Keywords
Contract farming, Opportunities, Side marketing, Smallholder farmers, Tobacco production

## Introduction
Since the inception of the tobacco credit facility there has been great controversy each marketing season between contract tobacco farmers and contracting companies over producer prices. Contract farming refers to the contractual arrangement between farmers and a firm, whether oral or written, specifying one or more conditions of production and marketing of agricultural products (Stringfellow, 1996). Watts (1994) referred to contract farming as the relationship between farmers and private or state enterprises that substitute for open-market exchanges by linking nominally independent family farmers of widely variant assets with a central processing, export, or purchasing unit that regulates in advance price, production practices, product quality, and credit. IFAD (2003) also notes that contract farming is the agricultural production governed by an agreement made between a buyer and a producer regarding the production and marketing conditions of one or more agricultural products. Smallholder farmers are small-scale farmers who manage farming activities varying from less than one hectare to 10 hectares. They are characterized by family-focused motives such as favouring the stability of the farm household system, using mainly family labour for production and using part of the produce for family consumption and a few surplus for sell (Kurt and Johann, 2006).

Providing smallholder tobacco farmers with sufficient inputs and purchasing their products at viable producer prices well before the onset of the rainy season are probably the most essential factors of the farmers cropping programmes. In Zimbabwe smallholder

farmers face an acute shortage of resources and the capacity needed for maximum productivity and marketing (TIMB, 2011). Smallholder farmers have the land but are constrained with financial resources to purchase the inputs. Thus smallholder farmers mostly depend on contract farming. In 1992 the Zimbabwe government embarked on the World Bank's Economic Structural Adjustment Program (ESAP), which encouraged the deregulation of agricultural marketing. This period saw the commencement of liberalization programmes for the main agricultural products which increased opportunities for companies to become involved in contract farming (Owen, 2008).

The government of Zimbabwe ceased subsidizing agricultural inputs to farmers. The agricultural bank required collateral security before granting loans. Other institutions like the Farmers Development Trust, Kutsaga and TIMB also could no longer fully support farmers especially in terms of finance. In 2004/05 season the Zimbabwe Tobacco Association linked 1523 smallholder tobacco farmers nationally to Northern Tobacco, Tribac and Zimbabwe Leaf Tobacco which process and market tobacco products. In the 2013/14 season more than 90 000 farmers registered to grow the golden leaf, with 70% of them in contract farming. Contracting companies benefit from the input credit scheme by making profits from the farmers' produce that they export or process to add value before marketing (Dawes, 2009). Some companies increase their profits by buying produce from farmers at lowest prices and in turn export them at higher prices (Reach et al., 2007; Wang and Deglad, 2014). Contract farming contributes to both increased production and income for farmers (FAO, 2001).

The main potential advantages of contract farming include provision of inputs, access to ready markets, appropriate technology and conservation skills transfer (UFAD, 2003; Wang and Deglad, 2014). Major challenges confronting tobacco growers mainly comprise of; resource limitations, poor agricultural management practices, lateness of operations, seasonal variability, defaulting by both parties, untimely provision of insufficient inputs, stiff competition among buyers, unviable producer prices and side marketing by farmers (Kumar, 2006; Kurt and Johann, 2006; Watts, 1994). Contracting tobacco farming should potentially enhance productivity and profitability. However the magnitude and reliability of the profit needs to be ascertained especially in rural wards such as ward 27 of Shamva district. The aim of the study was to assess the opportunities of contract tobacco farming and its sustainability to smallholder farmers.

## Materials and Methods

### Research Area

This study was carried out in Ward 27, Shamva district of Mashonaland Central Province in Zimbabwe. The study area falls in agricultural ecological region IIb and it is a hot and dry region with temperatures ranging from 21$^{o}$C to 23$^{o}$C during the hot season (AGRITEX, 2014). During the cold season the temperatures decline to as low as 10$^{o}$C. Shamva district has an average rainfall of between 600mm and 900mm per annum (AGRITEX, 2014). Its altitude varies from 900m to 1000m above sea level. The study area is characterized by sandy and sandy loam soils which are favourable for tobacco production.

### Sampling and sampling intensity

A systematic sampling method was used to select a sample of research participants. Farmers were arranged in alphabetical order from the four selected villages in Ward 27 and every third farmer was considered as a research subject. From the target population of 110 smallholder farmers (as supplied by Crop department, Ministry of Agriculture) under contract credit scheme by various contracting companies in the ward, a total sample of 33 smallholder farmers were selected for the study. Thirty percent of the total contracted tobacco farmers were considered ideal to take part in the study.

### Data collection and analysis

Only primary data was collected. The study was conducted mainly through the use of structured questionnaires and semi-structured interviews. Semi-structured interviews were conducted with key stakeholders from Agriculture extension officers, tobacco companies, the local councilor and village heads. These people were interviewed for both their perceptions and support of the contract scheme. Specific issues that were asked about the tobacco contract credit scheme included timely provision of inputs, timely payment of produce during purchasing, and challenges faced by the farmers in the contract credit scheme. Descriptive statistics and the Statistical Package for Social Sciences (SPSS) were used to analyze data so as to assess the challenges and benefits of tobacco growing under contract farming. Tables and pie charts were used to present the data.

## Results and Discussion

### Demographic characteristics of the respondents

The results revealed that the majority of farmers involved in the tobacco input credit scheme were male adults accounting 93.9% of the respondents (Table 1). Female headed households were 6.1%. This shows that the majority of households were headed by males. This may be attributed to the amount of labour demanded by tobacco operations and women headed families could not take up the risks involved. During interviews the female headed families practicing tobacco farming confirmed that they inherited the enterprises from their male counterparts with full farming implements, draught power and skills. They also highlighted that they have children who could help them in the whole production process. Application of techniques introduced by management like ridging, fertilizing, transplanting, thinning, de-suckering, pests and disease control and curing are hard to cope with for women headed families (FAO, 2012; Silva and Rankin, 2013).

The mean household size was six. The modal age of respondents was between 36-40 years, (36.4%), indicating that the majority of contracted tobacco farmers were middle aged (Table 1). Farmers less than 30 years and above 45 years were the minority accounting for 9.1% and 12.0% respectively. According to the respondents, young people aged between 20 and 25 years were mainly not involved in tobacco production. Key informants cited that the main reason for young people refraining from growing tobacco was their poor resource base including skills in tobacco production. The modal age of tobacco growers was 36

to 40 years with 36.4%. Farmers in this age group were skilled and energetic to run around organizing for inputs and carrying out all the necessary activities and field operations. It was noted that at the age of above 45 years, the number of farmers involved in tobacco growing decline possibly due to the strenuous work involved.

The majority of the respondents interviewed had attained education upto primary school level (36.4%), while secondary education (18.5%), tertiary education (9.1%) and 36% had not attended to any formal school (Table 1). It therefore meant that most of the respondents were better off in terms of education hence the ability to understand contract terms given to them by contracting companies. The results reflected a high literacy level of about 64% (Table 1). It follows that the majority of respondents could read contract terms and instructions written on seed, fertilizer, herbicides and pesticides packs for proper field application procedures. Commensurate with the level of education, respondents were legible for training in record keeping and simple book keeping, an important factor for the farmers to be able to assess business profitability. Provided with enough inputs including irrigation facilities, farmers reported that they could fully utilize all the inputs supplied to them by contracting companies. Most farmers used knapsack sprayers incorrectly and the potential results included waste of costly crop chemicals, poor pest and disease control, plant injury and high levels of chemical residues on the harvested crop (Glover, 1992).

Table 1. Demographic characteristics of participants

| Characteristic | Percent |
|---|---|
| **Gender** | |
| Males | 93.9 |
| Females | 6.1 |
| **Age (years)** | |
| 26-30 | 9.1 |
| 31-35 | 15.2 |
| 36-40 | 36.4 |
| 41-45 | 27.3 |
| >45 | 12.0 |
| **Education** | |
| No education | 36.0 |
| Primary | 36.4 |
| Secondary | 18.5 |
| Tertiary | 9.1 |

### Contracting Companies and Services provided to farmers

The research findings show that Zimbabwe Leaf Tobacco had the largest number of contracted farmers (43%), followed by Northern Tobacco with 33% of the farmers and Chidziva Tobacco Processors with 24% of the farmers (Table 2). Respondents asserted that the major services provided by contracting companies were the supply of agricultural inputs and to some extent, working capital for labour hiring. During interviews respondents associated this variance in contracted farmers to customer care and commitment which were demonstrated by the former company. Farmers complained that these contracting companies were not providing tillage services which were a critical component for enhancing tobacco output. However farmers confirmed that seed, fertilizers and chemicals were supplied on time (Table 3). According to Kumar (2006), one key feature of contract farming is that it provide farmers with access to markets that would not otherwise have been available to them. To some extent, contract farming schemes have acted as growth poles in sugar schemes in western Kenya, tea schemes in Tanzania and asparagus scheme in Peru. All these schemes have performed well in terms of opening up underdeveloped areas of the countries in which they are located (Silva and Rankin, 2013).

Table 2. Contracting companies and Services Provided

| Name of company | Percent | Services Provided |
|---|---|---|
| Zimbabwe Leaf Tobacco | 43 | Agricultural inputs provision, technical extension services and working capital |
| Northern Tobacco | 33 | Agricultural inputs provision and farmer training |
| Chidziva Tobacco Processors | 24 | Agricultural inputs provision |
| Total | 100 | |

### Farmers' experiences and expectations with contract farming

About 51.5% of the respondents reported that they had 2-3 years' experience of tobacco growing, 9.1% had grown tobacco for less than 2 years while 39.4% had more than 4 years of contract farming (Table 3). All interviewed farmers asserted their intention to continue with contract tobacco production. The modal age of experience reported by farmers during interviews was 2-4 years with 51.5% (Table 3). About 18% of the farmers were motivated by the need to acquire training skills in tobacco production. Ideally contracting companies should equip farmers with managerial skills to enable them to plan other activities outside but linked to contract farming. This is in agreement with Minton (1986), who reported that extension services should be designed to provide learning effects to go beyond production of the contract crop. Results of the study showed the need for marketing services (6.1%) and funds for hiring labour (9.2%). The results also show that contract farming was indispensable in Ward 27 since there were no alternative sources of funding. This agrees with the findings by Wang and Deglad (2014) for developing countries which indicated that contract farming is the only available source of funding to rural farmers as banks demanded collateral security and title deeds which farmers did not have.

Table 3. Period of contract farming and farmers' wishes to continue with contract farming

| Period of contract farming (years) | Percent |
|---|---|
| Less than 2 years | 9.1 |
| 2-4 years | 51.5 |
| More than 4 years | 39.4 |
| Total | 100.0 |

The researcher explored the reasons why farmers chose to continue with contract farming and the results revealed the following reasons. Farmers emphasized that they were motivated to continue with contract tobacco production for the cheap acquisition of basic agricultural inputs (52%), training in tobacco production (21%) while 18% indicated the need to secure markets (Figure 1). Only 9% of the respondents indicated the need for the working capital for hiring field labourers.

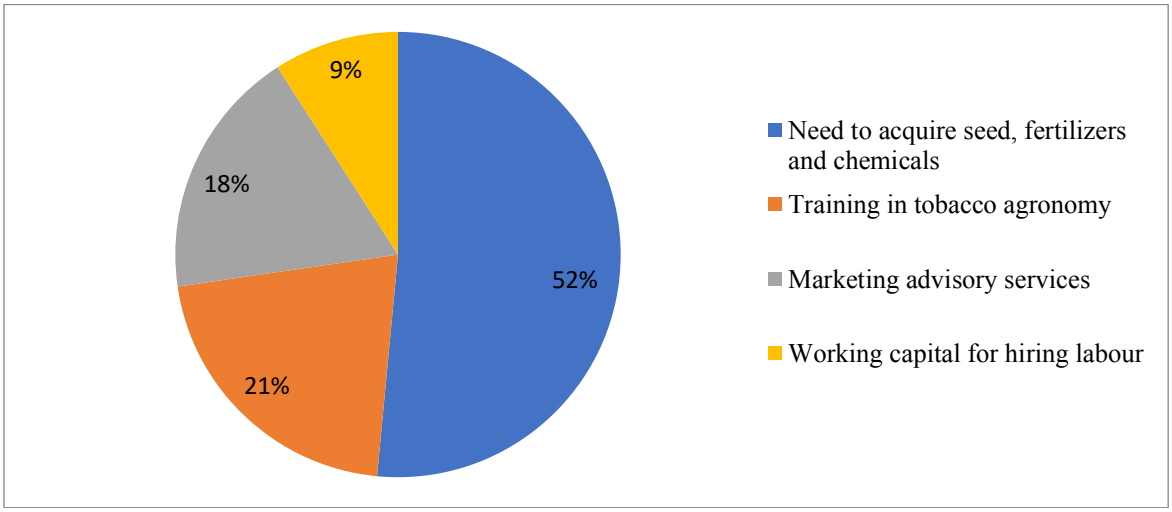


Figure 1. Reasons for continuing with contact farming

### Services provided by contracting companies

The study showed the different types of services which were received during the 2018/19 agricultural season and the responses given by the farmers are depicted in Table 5. The results revealed that 66.7% of the tobacco growers in ward 27 received seed, fertilizers, pesticides and herbicides during the 2018/19 agricultural season and 6.1% of them received advisory marketing services from the contracting companies (Table 4). The majority (85%) of tobacco farmers reported that inputs and other services were supplied well on time for the subsequent cropping season. Zimbabwe Leaf Tobacco and Northern Tobacco, in addition to supplying inputs, trained their growers in tobacco production (Table 2). Training was timed with field operations especially sowing of seed. Only Zimbabwe Leaf Tobacco provided working capital during the peak field operations which demanded additional labour. Some tobacco companies are unique in that they provide funds for hiring labour at certain times of the crop cycle (Kumar, 2006). No tillage services were provided by contracting companies. This implied that companies do not consider the importance of deep ploughing, let alone other soil management techniques like use of organic manures and soil pH moderation. These factors alone could also contribute to low productivity since continued use of inorganic fertilizers could cause soil acidity and pans which significantly reduce plant growth (Sartorius and Kirsten, 2006).

Table 4. Types of services farmers received from contracting companies during the 2018/19 agricultural season

| Services provided to farmers | Percent |
|---|---|
| Seed, fertilizers, herbicides and pesticides | 66.7 |
| Training | 18.0 |
| Working Capital for hiring labor | 9.2 |
| Marketing services | 6.1 |
| Total | 100.0 |

All the interviewed tobacco growers reported that tobacco producer price was determined by contracting companies alone. Farmers complained that they were not consulted during the producer price calculations. However, farmers appreciated that they were fairly paid on time for their produce.

### Challenges faced by farmers under tobacco contract farming

Fifty five percent of the respondents revealed that the greatest challenge faced by contract tobacco farmers was low producer price. High costs of inputs accounted for 30% while bad weather had 9% and shortage of fire wood 6% (Figure 2). The results denoted that contracting companies do not include farmers in the price determination of tobacco, citing the reason that prices were dictated by fluctuating prices prevailing at the world market at the time of selling (Table 5). These results reflected that contracting companies are monopolizing and manipulating the unfortunate situation of tobacco farmers of lacking resources and funding sources for tobacco production. The high cost of production and uncompetitive producer prices imposed by contracting companies to tobacco farmers renders the credit scheme unviable and unprofitable especially to the farmers. Da Silva and Rankin (2013) posits that the most significant income increases have been generated in those contract schemes in which smallholder farmers gain access to lucrative export markets for labour intensive luxury crops.

Measures to improve the financial viability of contract farming schemes should be monitored including setting appropriate pricing policies, rewarding risk taking by private companies in new crops and improving the autonomy and accountability of parastatals (Eaton and Shepherd, 2001). These findings contradicted with the study by Glover and Kusterer (1990) in Seke where Selby Enterprises negotiated the selling price of baby corn with farmers before contracting them and renegotiated the prices in the wake of threatening competitors. This company also advised its growers when prices were likely to be highest. In fact it is suspected that some buyers organized themselves in order to steal from companies (Dawes, 2009; Kessides, 2002). In some instances, the price at which the produce is bought might be so low that the farmers are not able to recoup the cost of transport they could have incurred, let alone recovering production costs.

Some payments were made late during floor auction days due to pressure at the auction floors. Some respondents reported that they had to go overnight awaiting payment. They cited inconveniences experienced during sleeping outdoor as well as risks of being conned by Harare men. These results pointed to the need for government to subsidize inputs to reduce production costs, intervene during price determination by companies and decentralize tobacco marketing services to production areas since farmers were having problems during transporting their tobacco to Harare and losing their hard earned cash to conmen. Where feasible project authorities should sign contracts and disburse payments at household level.

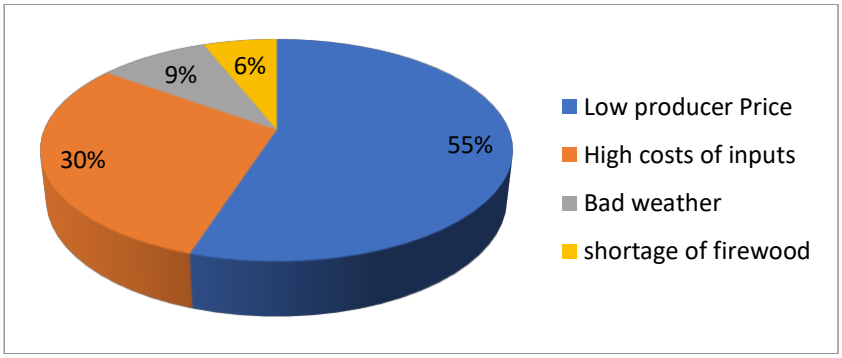


Figure 2. Challenges faced by farmers on contract credit scheme

### Challenges faced by contracting companies

The major challenges faced by the contracting tobacco companies ranged from defaulting on loan payment (40%), side marketing (27%), and inputs diversion and selling by farmers (17%) (Figure 3). However, competition among buyers (13%) and yield fraud (3%) were also indicated as challenges faced by contracting companies. Low producer price was attributed to the exclusion of tobacco farmers during price determination by contracting companies. Contracted farmers sometimes sell or divert fertilizers meant for tobacco production to maize crop which results in low productivity and low income (TIMB, 2011). Company officials argued that the cost of inputs were high since they included transport. This fact was also supported by Da Silva and Rankin (2013) who

noted that the transport costs were built into the producer price such that farmers took it as free service. Tobacco is harvested and cured starting from February. This means that bans, sheds and storerooms of farmers should be intact to avoid loses through tobacco spoilage by the rains. Carlos and Marlo, (2013) highlighted the need for companies to consider funding infrastructure to prevent post-harvest loses especially to beginners. Charcoal availability should be organized for tobacco curing to avoid cutting down of trees (Mutenje and Mango, 2019).

Contracting companies should consider decentralizing buying and processing of tobacco in the production areas to reduce transportation costs to the farmers, increase producer price and lure more farmers into the tobacco industry (IFAD, 2003; Kessides, 2002). The companies would in this way increase raw materials which they need most for processing or exporting. This would resultantly increase their sales income. In a study by Murko (2009), on contract farming in Thailand, Malaysia and Philippines, found out that farmers with very low yields were unable to repay their loan input scheme and when faced with the prospect of indebtedness they would often choose to sell the output elsewhere. According to respondents, other reasons that cause farmers to side market their produce include late payment by companies, greed and the feeling that companies were profiteering especially when the producer prices offered by contracting companies were lower than those of competing buyers.

While companies blamed farmers for selling inputs and diverting fertilizers meant for tobacco production to maize crop. Tobacco farmers argued that companies should provide farmers with full input scheme including tillage services. Inputs delivered for use on the contract crop are occasionally diverted to food crops depending on the farmers' estimates of relative marginal returns. Several aspects of contract farming impinge on food production and consumption by farmers, their families and employees and other segments of the population (Kurt and Johann, 2006; Singh, 2003). These findings showed that farmers recognized the importance of food and wanted to avoid buying it using funds from the cash crop like tobacco.

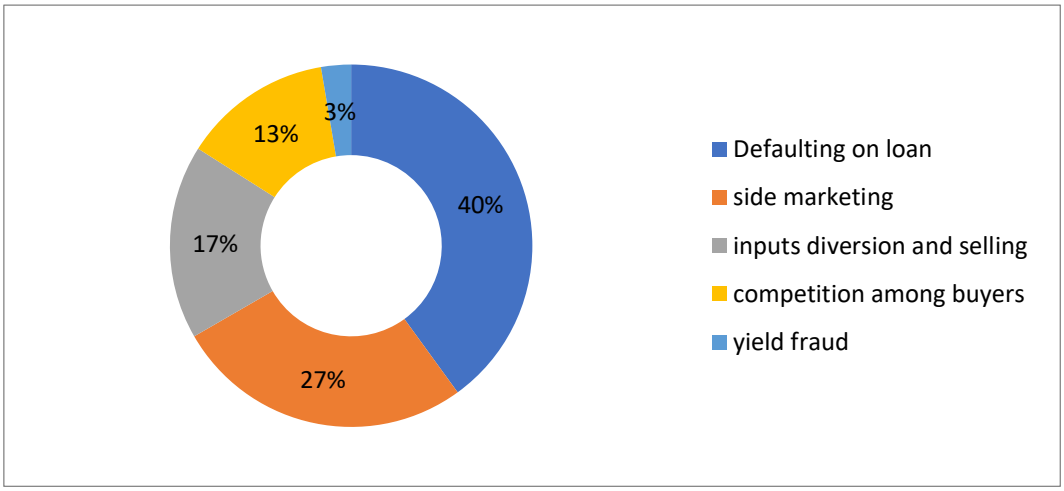


Figure 3. Challenges faced by contracting companies

### Challenges of contract farming as given by the Agriculture extension officers and the local leadership

Village heads echoed the problem of felling down of trees for fire wood by tobacco growers (57.1%) while agriculture extension officers were worried with farmers who were contracted yet they had no draught power, enough skills and labour force (28.6%). The councilor noted that the training of farmers is an important factor that would reduce the defaulting of loan payments, side marketing and misappropriation of inputs meant for tobacco production. About 57.1% of these respondents indicated that tobacco farmers' major challenge was shortage of fire wood as farmers could cut down big trees in order to cure their golden leaf. About 26.6% of the interviewees highlighted that some farmers had no draught power, enough skills and labour. Lack of tractors and farming implements can delay land preparation and potential crop yields diminish with late planting. Shortage of labour was also highlighted and this concurs with the findings by Ruttan (2007) who reported that farmers at Mkwasine Sugar Estate complained about severe labour shortages. The traditional labour pool had dried up as people sought better livelihoods in Mozambique or in informal trading and mining operations. In addition, the inability of contracting companies to wean off capable tobacco farmers may promote dependency syndrome among farmers.

### Solutions to challenges faced in contract farming

The inclusion of tobacco farmers during price determination by contracting companies was found to be the great remedy (38%). Training by both Agriculture extension officers and contracting companies accounted for 24% of the respondents and timely supervision of field operations had 24% as imperative solutions for a successful contract farming program (Table 5). Provision of tillage services and working capital were also suggested as contributing factors to the farmers' success by 14% of the respondents. Farmers highlighted that companies should provide tillage services and funds for labour hiring in addition to other agricultural inputs. The respondents wished that the government through the Ministry of Agriculture be involved by providing

irrigation infrastructure, and regulation of producer prices. Key informants from Agriculture extension officers also supported that tillage services should be provided to farmers to enhance planting on time and improve weed management. All stakeholders agreed that all the training services should be done by the Agriculture extension officers in conjunction with contracting companies to eradicate risks and uncertainty of crop failure. This in turn would enable farmers to utilize all the supplied inputs in their fields without diverting or selling some. Farmers can also repay all their debts and continue with their production of tobacco in mutual understanding with their sponsors (Glover, 1984). Agriculture extension officials, councilor and village heads in ward 27 reported unfair risk sharing between farmers and contracting companies citing that greater risks were apportioned to the farmers. Accordingly, they felt that these farmers should be weaned off from contracting companies and self-manage their production enterprises after certain years of input assistance.

Table 5. Solutions to challenges faced in contract farming

| Solutions to challenges | Percent |
|---|---|
| Training in record keeping and accounting | 24 |
| Timely supervision | 24 |
| Inclusion of farmers during price determination | 38 |
| Companies to provide tillage and working capital to famers | 14 |
| Total | 100 |

## Conclusions

The study was effective in its assessment on the challenges faced by tobacco farmers in the contract farming scheme. The low producer price, high costs of inputs, bad weather, and shortage of firewood for curing were cited as major challenges in tobacco contract farming. Similarly, contracting companies cited defaulting of loan by farmers, side marketing, diversion of inputs and yield fraud as major challenges in their own assessment to productive tobacco yield. In conclusion, the immediate solutions to these challenges included: training of tobacco farmers in record keeping and accounting, timely supervision and inclusion of farmers during price determination. While tobacco producer prices are not commensurate with production costs because prices are determined by contracting companies without involving the farmers the production risks are shouldered by the tobacco farmers.

## Recommendations

Farmers should be educated on financial management skills such as record keeping, labour costs and simple bookkeeping to enable them to assess profitability of their enterprises. These skills help instill financial discipline among farmers and allow them to be more vigilant in avoiding too many overheads. It is further recommended that researches on how large scale tobacco production impact on poverty reduction or prosperity gains be conducted to establish the challenges contracting companies encounter. There is also need to compare and contrast how other farming sectors are attempting to extend support to farmers. Nonetheless, contracting companies should offer tillage services as well as some funds for hiring labourers.

## Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

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

### Consent for publication

As authors of this manuscript, we have agreed that the paper be published with your journal.

### Ethical approval

Ethics committee approval is not required.

### Funding

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### Data availability

Not applicable.

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Zulkadir, G. (2020). Possible effects of priming on germination performance of white clover (Trifolium repens L.) seeds in hypothermia condition. Int. J. Agric. Environ. Food Sci., 4(1), 1-6.

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In "Possible effects of priming on germination performance of white clover (*Trifolium repens* L.) seeds in hypothermia condition. (Int. J. Agric. Environ. Food Sci., 4(1), 1-6 (2020))" the Author noted one eror.

**The Author contribution has been changed from:**

Author contribution

The idea, concept and methodological approaches of this research were determined by Prof. Dr. İskender TİRYAKİ. The author collected the data under supervision of Prof. Dr. İskender TİRYAKİ. The author prepared the manuscript and published it by taken the permission of Prof. Dr. İskender TİRYAKİ. The scientific responsibility of the published paper solely belongs to author per se.

The correction will appear in the online version of the paper on the nternational Journal of Agriculture, Environment and Food Sciences (Int. J. Agric. Environ. Food Sci.) website on 6 (1) March 2022 Issue, together with the publication of this correction notice.

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Research Article

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# Possible effects of priming on germination performance of white clover (*Trifolium repens* L.) seeds in hypothermia condition

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**Abstract**

The aim of the study is investigate the effects of hidropriming on germination performance of white clover (*Trifolium repens*) seedsunder cold stress, optimum temperature and dark condition. The seeds were germinated applying hidropriming with $dH_2O$ in different periods (2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) in dark at 4 °C. The study was applied in according to randomized controlled trials as 4 replications to measure radicular lengths, germination rate and homogeneity parameter in the seeds. The results of the study was indicated that radicle length, germination rate, rate and the homogeneity parameter were positive and statistically significant ($P<0.001$) comparing with control group in short period (2 and 4 hours). On the other hand radicle length, germination rate, the homogeneity parameter were found negative and statistically significant when compare with control group in the other periods ($P<0.001$). In addition, this study was discussed possible physiological effects to the priming process implemented with $dH_2O$ in hypothermia condition at white clover seeds.

**Keywords:** Cold stress, Germination, Hidropriming, White clover

## Introduction

White clover (*Trifolium repens*) which widely cultivated in the Marmara, Black Sea and transition zones in Turkey is quite valuable for animals. This plant which is short, abundant leaf, thin handle, soil surface covering, and high quality production is a high quality meadow pasture and forage crop (Manga et al. 1995). Germination of plants varies between varieties but it is highly influenced by environmental factors such as light, water, temperature, oxygen etc. Water and temperature factors are the most commonly used environmental factors investigated to start germination of the seeds. Theoretically, it is possible to stimulate the germination of the seeds with these applications to get fast and high germinate percentage (Karakurt et al. 2010). For this purpose, humidification / hydropriming applications on plants have demonstrated beneficial effects on the rate of germination such as Basu and Pal (1980) in rice seeds, Rao et al. (1987) in lettuce seeds, Sivritepe and Dourado (1995) in pea seeds, Sivritepe and Demirkaya (2002) in onion seeds, Demirkaya (2006) in Çetinel-150 pepper seeds (Karakurt et al. 2010). Özdemir (2006) observed a positive effect on the germination rate of the kiwi (Hayward varieties) seed with low germination rate by applying hdropriming with pure water at different temperatures and different durations.

The damage of plants due to low temperature has been seen varies between species and variety. According to Oquist (1983), low temperature is a relative expression. Plants adapted

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to cold climates may photosynthesize at temperatures below 0 °C and may develop normally, while the photosynthesis mechanism may undergo irreversible damage at temperatures below 10 °C in tropical and semi-tropical climatic plants. According to Raison and Lyons (1986), the term cold damage defines the physiological damage that occurs when most tropical and semi-tropical plants are exposed to a low but not freezing temperature (usually up to 15 - 0 °C). Inhibition of photosynthesis is a precursor reaction to low temperature. The effect of cold damage photosynthesis is increases with medium or high light intensity. The damage to the cold is partly increased by free oxygen radicals that cause secondary damage to membranes and photosystems (McKersie and Leshem 1994). The increase in electrolyte output with membrane damage in tissues exposed to low temperature is the most commonly known effect of low temperature (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985). Rylski (1973) in his study on pepper has been reported the flowering time and growing and development time of vegetative organs in plants increased with the decrease in air and soil temperature. Polowick and Sawhney (1985) conducted a study on Vinedale type. According to results, the low temperature (18 °C day / 15 °C at night) cause functional male infertility by abnormal such as petal leaf, male organ and female organ formation, male organ deterioration, in some cases partially carpel-like male organs have the ability to abnormal flower powder production. Researchers who determine that the ovary is larger than normal and the style extension is prevented were reported plants that grow at low temperatures produce small fruits, which are seedless, but are pollinated with flower dust from plants growing at normal and high temperatures. Aloni et al. (2001) reported that low temperature affected the carbohydrate mechanism negatively. It negatively affects the characteristics of the pollen and the germination rate and also accordingly fruit shape, shape and development are affected (Shaked et al. 2004). Farrell et al. (2006) reported that low temperature application on rice decrease the amount of flower powder and starch it accumulates. In the study on chickpea of Nayyar et al. (2005), low temperatures on the reproductive organ causes abortion and flowering and fruit attitude were negatively affected. During cold stress, due to disruption of the membrane function (cytoplasmic, mitonodrial and chloroplast membrane), photosynthesis is reduced, carbohydrate transport slows, respiration slows, protein synthesis is inhibited, the rate of disintegration of existing proteins increases, the dissolved substance exudes from the cell. Low temperature affects the activity of integral membrane proteins that regulate the transport of H+-ATPaz' carriers, enzymes, ions and other dissolved contents of required for metabolism (Taiz and Zieger 2008). In cold sensitive plants, the double layer oils have a high proportion of saturated fatty acid chains. This type of membrane reaches the semi-crystalline phase at temperatures above 0 °C. As the membrane loses its fluid, the protein components are no longer working. The membrane lipids of cold-resistant plants have generally higher unsaturated fatty acids than cold-sensitive plants (Kaçar et al. 2006). A gene was transferred from Escherichia coli to Arabidopsis in order to increase the high-melting (saturated) membrane lipids. This gene has greatly increased the resistance of gene-modified plants to low temperature (Taiz and Zieger 2008).Moran et al. (1989) have studied that effect of the study of growth and root growth of lentil (*Lens culinaris Medik*. Cv. Castellana) plant under water and alternative temperature (4-20 °C, 16/8 hours). According to this study, they determined that the osmotic potential was initially high but it was lower controls than in the alternative temperature regimen. The cellulose content of the stem cell wall was similar in all applications on the 3rd day of development, but lower than the controls. In all cases during the study period, natural non-cellulosic sugars in the cell wall decreased but in stressful roots were higher than controls. While uronic acids and protein content were higher in roots at control groups, the amount of uronic acids increased and differences of protein content was not found significant.

In this study, the application of hydropriming and the times of this application were investigated of the effects of rate and speed of the seed germination besides the development of the plant under the cold stress.

**Materials and Methods**

In the study, white clover (*Trifolium repens*) seedswere used as trial material. The study was carried out in the Department of Field Crops, Faculty of Agriculture, Kahramanmaras Sutcu Imam University.

**Hydro-Priming Application**

White clover seeds used as test material were soaked with dH2O at different times (0, 2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) and 4 ± 1 ºC in dark condition. Then, all seeds taken to the drying paper in the room conditions. The main aim in there is to remove the water from the seed surface. The dried seeds were placed in glass petri dishes (5x5.5 cm) in which two layers of drying paper.

Petri dishes with seeds was added 3 ml of distilled water and then these were germinated in a climatic cabinet at 15 ± 1 ºC temperature and in the dark condition. Also, untreated

seeds were used as control seeds. The study was carried out with 4 replications according to the experimental design of randomized plots.

In this study, germinated seeds were counted daily and removed from petri dishes and then their radicle length was measured. This process continued until the number of seeds germinated in all petri dish was zero for 3 consecutive days (9 days). In the experiment, 1-2 mm root outlet was accepted as germinated seed.

This study was examined traits such as percentage of germination, angular transformation of germination percentage, time to germinate 50% of seeds (day), time to germinate 90% of seeds (day), time for germination of 10% of seeds (day), span value (the number of days required for seeds to reach 90% germination by 10%), total radicle length, average radicular length.

**Statistical Analysis**

The last germination percentage (GerY) and its angular transformation (arcsine√GerY) and also the root length of germinated seeds were determined in germinated seeds. Then, the data of obtained were analysed by SAS (SAS 1997) statistical package program. The differences between the means were tested by Fisher's smallest significant difference (LSD) test at grade of $p < 0.05$.

**Results and Discussion**

The results of the research have shown Table 1 and Figure 1-2 belong to white clover seeds which are germinated after hydropriming application at different times under cold stress. When the results were examined, it was observed statistically significant differences between the seeds applied of hydropriming at different times under cold stress. Accordingly, it has been observed a small but continuous decrease due to increased hydropriming time at the germination rates. It was determined that the highest germination rate with 95% was obtained from the seeds which were applied hydropriming for 0 and 2 hours while the lowest germination rate was obtained from the seeds applied hydropriming for 48 hours with 37%. The germination rates of the seeds which were applied hydropriming for other periods (4, 6, 8, 16, 18, 20, 22 and 24 hours) were 82%, 78.5%, 68.5%, 68%, 56%, 54.5%, 49.5% and 57.5%, respectively (Fig. I).

The highest root length value was obtained from seeds which were applied of hydropriming for 2 hours with 176 mm. Total root length of the seeds of control (0 hour) was obtained 149.5 mm. In the other periods (4, 6, 8, 16, 18, 20, 22, 24 and 48 hours), the total root length values from seeds which were applied of hydropriming were determined as70.75, 48.50, 44.50, 40.75, 33.50, 30.50, 32.25, 31.75 and 20.00mm, respectively (Fig. 1). At the application of hydropriming for 4 hours and more time, there have been significant decreases in the proportions total root lengths. According to obtained data, the ratio of the total root length to the total number of germinated seeds and the average root length per seed germinated (ARL) were calculated. When the ARL data were compared between the applications, the maximum root length was determined as 1.85 mm and 1.73 mm values from seeds which were applied of hydropriming for 2 hours and 4 hours , respectively, while this value was determined as 1.57 mm in the control seeds. The ARL data for other applications (6, 8, 16, 18, 20, 22, 24 and 48 hours) were determined as 1.24, 1.30, 120, 1.20, 1.12, 1.30, 1.10 and 1.08 mm, respectively (Figure 2).

At the seeds which were hydropriming applicated at different times under cold stress, germination parameters such as speed of germination, TRL, ARL and germination rate of seeds were found different ($p<0.01$). The most rapid germination (G50 = 1.55 days) in seeds germinated depending on the duration of the application was obtained from the seeds the applied of hydropriming for 2 hours. In addition, this value was determined as 1.67 days in any untreated control seeds. In the seeds germinated depending on the application time, the slowest germination was determined as 2.52 (for 22 hours), 2.50 (for 18 hours) and 2.47 days (for 20 hours), respectively. In other applications, the speed of germination which 4, 48, 16, 8, 6 and 24-hours applications was determined to 1.74, 1.85, 2.09, 2.13, 2.14 and 2.20 days, respectively. According to these results, the other times except for 24-hour application showed slower germination than control and the 2-hour application, it was determined that the germination rate was slowed continuously with little difference in increasing time. But the 24-hour application showed faster germination than 18, 20 and 22 hours application.

In the light of this data, the highest values of all germination parameters were obtained from the seeds which were subjected to hydropriming for 2 hours, and then were obtained from the seeds which were subjected to hydropriming for 4 hours. When compared with the control group, it was determined that these applications were higher than the control group. It was determined that the other applications had less but gradually decreased values in the control group.

The purpose of the application of hydropriming was to provide water transition to inside of the seed to start germination. In this study, we used hidropriming of applications under cold

stress (4 ºC) and applicated different time periods in order to determine how it promote germination. According to these results, the 2-hour hydropriming application caused an increase in speed of germination, germination rate, TRL and ARL values compared to control. However, we found that the other applications decreased continuously. The germination and development in seeds is slowing due to $O_2$ deficiency in excess water stress. However, it changed death in plants according to the duration of exposure to excessive water stress. On the other hand, seed and plants under cold stress are getting slowing water intake and damage due to $O_2$ shortage usually occurs in plants after 24 hours depending on the variety and species (Taiz and Zieger 2008). This situation made us think that this hydropriming application under cold stress did not cause any damage due to O2 scarcity in seeds. The white clover seeds which the most suitable germination temperature of 20-25 ºC were determined that didn't adversely affect the seeds up to 4 hours in temperature of 4 ºC.

Even, it has been observed that hydropriming positively promotes germination but the negative effects of the 4 ºC temperature on the germination. And root growth in the white clover seeds were found to be lower gradually after 4 hours. In the light of previous studies by many researchers, this situation has been disclosed as increase in electrolyte output by membrane destruction in tissues exposed to low temperatures (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985), negative effects on carbohydrate metabolism (Aloni et al. 2001), decreased photosynthesis, slow down transport of carbohydrates, slowing of respiration, inhibition of protein synthesis, increasing the rate of fragmentation of existing proteins, leakage of dissolved substances from cells, the physical properties of the lipids involved in the membrane structure affect the activity of H+-ATPaz' carriers, integral membrane proteins that regulate the transport of enzymes, ions and other dissolved substances necessary for metabolism (Taiz and Zieger 2008), osmotic potential during root growth and reduction of cellulose content of root cell wall (Moran et al. 1989).

Table 1. Data on germination performance of white clover seeds of cold stress pre-treatment at different times

| Application | Germination Rate (GerY) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % | [GerY] | $G_{50}$ | $G_{90}$ | $G_{10}$ | Span | TRL | ARL |
| 0 hour | 95.00 | $61.22^{A}$ | $1.67^{CD}$ | $2.94^{DE}$ | $1.10^{AB}$ | $1.84^{E}$ | $74.75^{B}$ | $1.57^{B}$ |
| 2 hours | 95.00 | $62.97^{A}$ | $1.55^{D}$ | $2.62^{E}$ | $1.00^{B}$ | $1.62^{E}$ | $88.00^{A}$ | $1.85^{A}$ |
| 4 hours | 82.00 | $53.43^{B}$ | $1.74^{BCD}$ | $3.60^{CD}$ | $1.09^{AB}$ | $2.51^{DE}$ | $70.75^{B}$ | $1.73^{AB}$ |
| 6 hours | 78.50 | $51.80^{B}$ | $2.14^{ABC}$ | $4.11^{BC}$ | $1.14^{AB}$ | $2.97^{CD}$ | $48.50^{C}$ | $1.24^{CDE}$ |
| 8 hours | 68.50 | $48.17^{BC}$ | $2.13^{ABC}$ | $4.48^{ABC}$ | $1.03^{AB}$ | $3.44^{BCD}$ | $44.50^{C}$ | $1.30^{C}$ |
| 16 hours | 68.00 | $48.03^{BC}$ | $2.09^{ABC}$ | $4.12^{BC}$ | $0.91^{B}$ | $3.22^{BCD}$ | $40.75^{CD}$ | $1.20^{CDE}$ |
| 18 hours | 56.00 | $43.85^{CD}$ | $2.50^{A}$ | $4.99^{AB}$ | $1.19^{AB}$ | $3.80^{ABC}$ | $33.50^{D}$ | $1.19^{CDE}$ |
| 20 hours | 54.50 | $43.40^{CD}$ | $2.47^{A}$ | $4.78^{AB}$ | $1.31^{A}$ | $3.48^{BCD}$ | $30.50^{D}$ | $1.12^{CDE}$ |
| 22 hours | 49.50 | $41.83^{DE}$ | $2.52^{A}$ | $5.13^{AB}$ | $1.09^{AB}$ | $4.03^{AB}$ | $32.25^{D}$ | $1.28^{CD}$ |
| 24 hours | 57.50 | $44.28^{CD}$ | $2.20^{AB}$ | $4.85^{AB}$ | $1.06^{AB}$ | $3.79^{ABC}$ | $31.75^{D}$ | $1.11^{DE}$ |
| 48 hours | 37.00 | $37.44^{E}$ | $1.85^{BCD}$ | $5.19^{A}$ | $0.46^{C}$ | $4.73^{A}$ | $20.00^{E}$ | $1.09^{E}$ |
| p>0.001 | | ** | ** | ** | ** | ** | ** | ** |

**: significant at p <0.01; §, GerY: Percentage of germination; [GerY]: Angular transformation of germination percentage; G50: Time to germinate 50% of germinated seeds (day); G90: Time to germinate 90% of germinated seeds (day); G10: Time for germination of 10% of germinated seeds (day); Span: The number of days required for germinating seeds to reach 90% germination by 10%; TRL: Total radicle length of germinated seeds; ARL: Average radicular length of germinated seeds

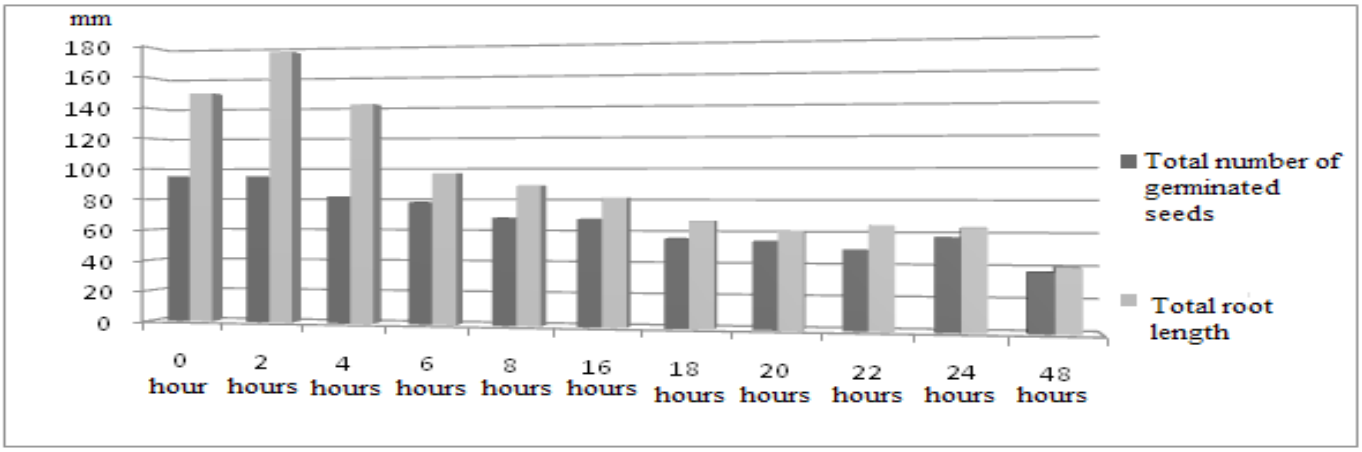


Figure 1. The means values of total germinated seeds and total root length data of germinated seeds

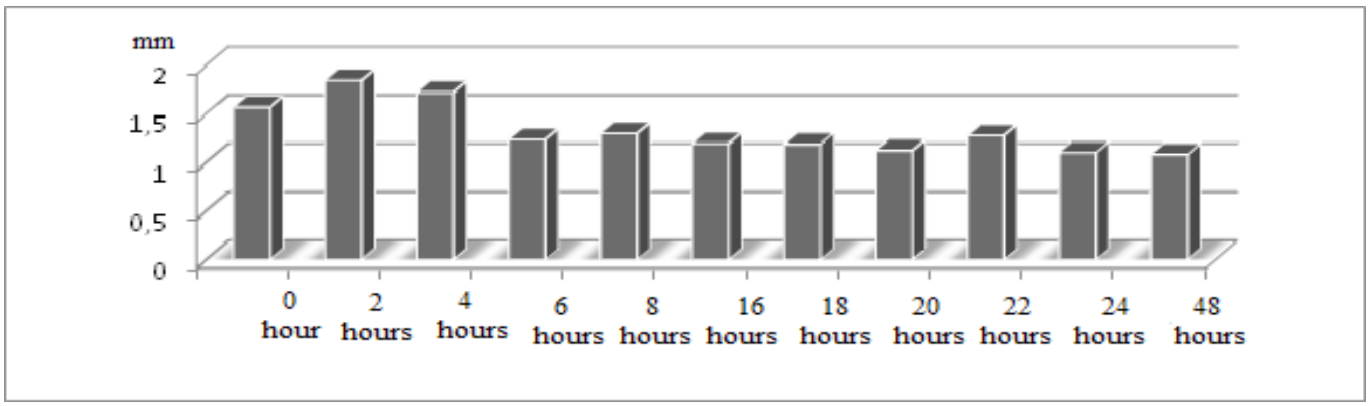


Figure 2. The means of average root length data per seed germination

**Compliance with Ethical Standards**

**Conflict of interest**

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

**Author contribution**

The idea, concept and methodological approaches of this research were determined by Prof. Dr. İskender TİRYAKİ. The author collected the data under supervision of Prof. Dr. İskender TİRYAKİ. The author prepared the manuscript and published it by taken the permission of Prof. Dr. İskender TİRYAKİ. The scientific responsibility of the published paper solely belongs to author per se.

**Ethical approval**

Not applicable.

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**Data availability**

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**Consent for publication**

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